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Master thesis:

Influences of the amino acids during alcoholic fermentation and development of aroma compounds in Sauvignon Blanc

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Erasmus+

Dedicated to my parents Graziella Di Mercurio and Giovanni Serughetti

Thank you for all the support along the way

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Abstract

In recent years, New Zealand in terms of production has expressed a certain respect and attention towards Sauvignon Blanc for its organoleptic chemical characteristics.

In this project seven different amino acids were used, two blend of amino acids and the DAP (di-ammonium phosphate) used in wineries, with the aim of investigating the effect of different levels of nitrogenous source of musts from the two different vineyards (Omaka Vineyard and Gifford's Creek Vineyard), on the progress of fermentation and the impact on the aromatic component, with the use of the "New Zealand Grape and Wine Research Program" winemaking protocols, with all the processes being reduced to improve the aromatic quality of Sauvignon Blanc in the Marlborough region of New Zealand.

This experimental thesis project with the title "Influences of amino acids during alcoholic fermentation and the development of aromatic compounds in Sauvignon Blanc", observes the single amino acids added starting from two musts, with different initial nitrogenous source, and examines the reaction from the yeast during alcoholic fermentation, with reference to the type of free aromatic compounds and their concentration.

The early harvest is a peculiarity of Sauvignon Blanc, characterized by its total acidity, in fact, there is an acidity of 14 grams per liter expressed in tartaric acid and for the normal harvest period about 11 grams per liter.

The soil in New Zealand has a great fertility, in fact, the producers convey the forms of pruning by concentrating a high production with more than 10 kilograms per plant and with a high production per hectare of about 23 tons.

Sauvignon Blanc represents 72% of the cultivated varieties, is recognized for its high aromatic quality and for the presence of thiols.

In fact, two thiols 3MH (3-mercaptohexan-1-ol) and 3MHA (3-mercaptohexyl acetate) have been described with the presence of pepper, gooseberry, passion fruit and tropical fruit, other notes include cut grass, tomato stalks, grapefruit and lime.

In 2016 the total production of grapes was 304,000 tons with 85% of wine exported.

Sauvignon Blanc is a variety that, working in reduction, releases thiolic compounds during alcoholic fermentation that play a fundamental role in the wine bouquet, and these aromatic compounds are very sensitive to oxidation, in fact, it becomes necessary to preserve these key compounds during the harvest, transport, winemaking and storage.

The research has focused on the use of two musts with different concentrations of nitrogenous sources, to study the production of aromatic compounds by yeast, with the use of different nitrogen sources, of which seven are single amino acids and two BLEND of amino acids and

the use of the usual DAP winery product, it has been found on the basis of the treatments that the aromatic compounds change in the wine producing in some high concentrations of esters, in other high concentrations of alcohols, this result has depended also from the initial YAN (yeast assimilable nitrogen) of the Sauvignon Blanc must.

Resumo

Nos últimos anos, a Nova Zelândia em termos de produção expressou um certo respeito e atenção ao Sauvignon Blanc por suas características químicas organolépticas.

Neste projeto utilizaram-se sete aminoácidos diferentes, duas misturas de aminoácidos e o DAP (fosfato de di-amônio) utilizado em vinícolas, com o objetivo de investigar o efeito de diferentes níveis de fonte nitrogenada de mostos dos dois vinhedos diferentes (Omaka Vineyard e Gifford's Creek Vineyard), sobre o progresso da fermentação eo impacto no componente aromático, com o uso dos protocolos de vinificação "New Zealand Grape and Wine Research Program", com todos os processos sendo reduzidos para melhorar a qualidade aromática de Sauvignon Blanc na região de Marlborough, na Nova Zelândia.

Este projeto de tese experimental com o título "Influências de aminoácidos durante a fermentação alcoólica e o desenvolvimento de compostos aromáticos em Sauvignon Blanc", observa os aminoácidos únicos adicionados a partir de dois mostos, com fonte nitrogenada inicial diferente e examina a reação do levedura durante a fermentação alcoólica, com referência ao tipo de compostos aromáticos livres e à sua concentração.

A colheita precoce é uma peculiaridade do Sauvignon Blanc, caracterizada pela sua acidez total, na verdade, há uma acidez de 14 gramas por litro, expressa em ácido tartárico e para o período normal de colheita, cerca de 11 gramas por litro.

O solo na Nova Zelândia tem uma grande fertilidade, de fato, os produtores transmitem as formas de poda concentrando uma produção elevada com mais de 10 quilos por planta e com alta produção por hectare de cerca de 23 toneladas.

Sauvignon Blanc representa 72% das variedades cultivadas, é reconhecida pela sua alta qualidade aromática e pela presença de tióis.

Na verdade, foram descritos dois tioles 3MH (3-mercaptohexan-1-ol) e 3MHA (acetato de 3-mercapto-hexilo) com a presença de pimenta, groselha, maracujá e frutas tropicais, outras notas incluem grama cortada, talos de tomate, toranja e limão.

Em 2016, a produção total de uvas foi de 304 mil toneladas com 85% de vinho exportado.

Sauvignon Blanc é uma variedade que, trabalhando em redução, libera compostos tiólicos durante a fermentação alcoólica que desempenham um papel fundamental no buquê de vinho e esses compostos aromáticos são muito sensíveis à oxidação, de fato, torna-se necessário

preservar esses compostos-chave durante a colheita , transporte, vinificação e armazenamento.

A pesquisa centrou-se no uso de dois mostos com diferentes concentrações de fontes nitrogenadas, para estudar a produção de compostos aromáticos por levedura, com o uso de diferentes fontes de nitrogênio, das quais sete são aminoácidos simples e duas MISTURAS de aminoácidos e o uso do produto da adega DAP usual, foi encontrado com base nos tratamentos que os compostos aromáticos alteram no vinho produzido em algumas concentrações elevadas de ésteres, em outras concentrações elevadas de álcoois, esse resultado dependeu também a partir do YAN inicial (nitrogênio assimilável de fermento) do Sauvignon Blanc deve.

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List of abbreviations:

Ala	**Alanine**
Arg	**L-Arginine**
Asp	**L-Aspartic Acid**
BLEND1	**mix amino acid 1**
BLEND2	**mix amino acid 2**
3MH	**3-Mercaptohexan-1-ol**
3MHA	**3-Mercaptohexyl acetate**
4MMP	**4-Mercapto-4-methylpentan-2-one**
°C	**Degree Celcius**
CTR	**Control**
CoA	**Coenzyme A**
SBMP	**3-sec-butyl-2-methoxypyrazine**
CO_2	**Carbon dioxide**
DAP	**diammonium phosphate**
FTIR	**Fourier transform infrared**
FS	**Free Sulphur**
g	**Gram**
GAE	**Gallic acid equivalents**
Gln	**L-Glutamine**
Glu	**L-Glutamic acid**
GSH	**Glutathione**
GC	**Gifford's Creek**
h	**Hour**
hl	**Hectolitre**
HPLC	**High-performance liquid chromatography**
kg	**Kilogram**
H_2S	**Hydrogen sulphide**
H_2O_2	**Hydrogen Peroxide**
H_2SO_4	**Sulfuric acid**
IBMP	**3-isobutyl-2- methoxypyrazine**
IPMP	**3-isopropyl-2-methoxypyrazine**
l	**Litre**
mg	**Milligram**

min	**Minutes**
ml	**Millilitre**
mm	**Millimetre**
NCR	**nitrogen catabolite repression**
N	**Nitrogen**
NH_4	**Ammonium**
NCR	**Nitrogen Catabolite Repression**
ng	**Nanogram**
nm	**Nanometre**
NOPA	**Nitrogen by o-phthaldialdehyde assay**
NTU	**Nephelometric Turbidity Unit**
NZD	**New Zealand Dollar**
OV	**Omaka Vineyards**
PFR	**Plant & Food Research**
ppm	**Parts per million**
PUFA	**Poly-unsaturated fatty acid**
rpm	**Rounds per minute**
S	**Sulphur**
Ser	**Serine**
SFA	**Saturated fatty acids**
SO_2	**Sulphur dioxide**
t	**Tonnes**
TA	**Titratable acidity**
TCA	**Tricarboxylic acid cycle**
UFA	**Unsaturated fatty acid**
V	**Volume**
wt.%	**Weight percent**
WHC	**(water holding capacity)**
YAN	**Yeast assimilable nitrogen**
µg	**Microgram**
µm	**Micrometre**

1. Introduction

Over the last few years, New Zealand is becoming a premium wine with great production of Sauvignon Blanc for its great and famous acid that in the early harvest are about 14 grams of litre and normal for about 11 grams of litre. And the soil has a great fertility, where producers push the plant to a high production with more than 10 kilograms per plant and with high production per hectare talk about 23 tonnes.

Especially when talk about New Zealand talking about Sauvignon Blanc is having great standards in the territory of New Zealand about 72%.

Sauvignon blanc is acknowledged for its high aromatic quality, with the presence of methoxypyrazines and thiols, the two most important thiols that have been related are 3MH and 3MHA with the greatest presence capsicum (bell pepper) and gooseberry characters through lush passion fruit and tropical fruit overtones, other notes include fresh cut grass, tomato stalks, grapefruit or limes.

The New Zealand wine industry has a target of $2 billion of exports by 2020. In the vintage 2016, the value of exports lifted 10% to just under $1.6 billion, the 21st consecutive year export value has grown (New Zealand, Winegrowers, 2017).

In 1973, as Marlborough's first Sauvignon Blanc vines were being planted, no one could have predicted that this variety would attain superstar status within a couple of decades. The explosive flavours of New Zealand Sauvignon Blanc have dazzled wine critics throughout the world, setting the international benchmark for the style.

In New Zealand are present this variety: Sauvignon Blanc, Chardonnay, Pinot Noir, Merlot, Cabernet Sauvignon, Syrah, Riesling, Pinot Gris, Gewürztraminer, with major production of Sauvignon Blanc in particular in Marlborough region, and Central Otago for Pinot Noir.

In 2016 the total production did 304.000 tonnes with 85% proportion of NZ wine exported.

Sauvignon Blanc have aroma compounds of the thiols that is group play an important role, and this aroma compounds are so much sensitive to oxidation, is important to preserve these key compounds during harvest, transportation, winemaking and storage. There are a lot research about this aroma compounds, but in this project was use seven different amino acid, two blend and DAP is a usual product in the winery and to investigate the effect in two different vineyard Omaka Vineyard and Gifford's Creek different levels of juice nitrogen/nutritional status on fermentation rates and the impact on flavour, for use in research scale winemaking protocols for the New Zealand Grape and Wine Research Programme, and monitoring alcoholic fermentation all process in reduction and to see the development aroma compounds production from yeast using the Sauvignon Blanc Marlborough Region.

This Master's Thesis project with title "Influences of the amino acids during alcoholic fermentation and development of aroma compounds in Sauvignon Blanc", Allow to

understand which amino acid predominates during alcoholic fermentation and which is more important in terms of aromatic composition.

2. Bibliographic review:

New Zealand Sauvignon blanc, specifically Marlborough SB, has become recognized and appreciated internationally by wine consumers and critics due to its distinctive tropical style. This wine variety is considered New Zealand's flagship wine as it constitutes more than 80 % of export wine (New Zealand Winegrowers 2012). Three varietal thiols are particularly important for SB wines: 3-mercaptohexyl acetate (3MHA), 3-mercaptohexan-1-ol (3MH) and 4-mercapto-4-methylpentan-2-one (4MMP), which account for the passionfruit, grapefruit and box tree (cat's pee) aromas respectively (Tominaga et al. 1998a; Benkwitz et al. 2012), Although other compounds add to the flavour profile like esters, C6 compounds and fusel alcohols, not all contributors to Sauvignon aroma are known now (Aznar et al., 2003). It is believed that these varietal thiols are initially not present in grape juice and develop during fermentation by the action of yeasts on juice precursors (Tominaga et al. 1996, 1998a, b). However, recently, very small amounts of 3MH (100 ng/L) have been detected in grape juice (Capone et al. 2011), which might have been produced in the grape juice during juice manipulation by the action of natural yeasts or other addition.

Marlborough Sauvignon blanc shows the highest analytical rates of methoxypyrazines and thiols (3MH and 3MHA), when compared to wines, coming from France, South Africa and Australia. Australia Sauvignon blanc are known to have apple, and lolly characteristics while South African and French wines show general notes of minerals, flintstone and bourbon (Lund, et al. 2009).

Samuel Marsden planted the first grape vines in 1819 in Keri Keri in New Zealand (Scott, 1964). It took more than 150 years before New Zealand's wine growers and wine makers started producing internationally acknowledged wines. In 1974, Matua Valley Wines produced the first trial wine from Sauvignon Blanc at Waimauku, near Auckland, in 1979; Montana released its first Sauvignon Blanc from Marlborough and commercially established that variety (Cooper et al., 1996). In the 1980s Montana's piercingly herbaceous, a more subtle and riper Cloudy Bay and Hunter's wines were responsible for alerting the world to the unique quality of New Zealand Sauvignon Blanc (Cooper et al., 1996). Sauvignon Blanc wines were single-handedly responsible for introducing New Zealand wine to the world. In particular, Sauvignon Blanc from the Marlborough region located at the top of New Zealand's South Island is known for its own distinctive style. Marlborough Sauvignon Blanc is intensely fragrant, more obviously fruity than the Loire prototype, with just a hint of both grass and sweetness and, occasionally, gooseberries and asparagus. That blend of tropical fruit flavour (melons, pineapple and

passion fruit) coupled with gooseberry and capsicum based herbaceous character is usually regarded as New Zealand's national Sauvignon Blanc wine style (Robinson, 2006).

The distinctiveness of Marlborough Sauvignon Blanc can also be attributed to the different winemaking techniques utilized by New Zealand winemakers when compared to Sauvignon Blanc produced in the old world (Sancerre and Loire) (Lund et al., 2009; Nicolau et al., 2006).In France, the use of oak barrels in quite common with Sauvignon Blanc whereas in New Zealand, winemakers choose to employ simpler techniques focusing on enhancing varietal fruity characters rather than aiming for complexity and longevity (Lund et al., 2009; Nicolau et al., 2006). Fermentation is 16 carried out in stainless steel tanks usually at low temperatures (12-18^{0}C). These winemaking techniques together with viticultural practices, soil and climate are thought to be responsible for the unique New Zealand style of Marlborough Sauvignon Blanc.

2.1 Amino Acid:

Nitrogen composition of the must has an important effect on rate of fermentation because nitrogen compounds increase biomass production and stimulate the rate of sugar utilization avoiding block of fermentation. Moreover, the amino acid are precursors of volatile compounds, in fact in really important talk about the nitrogen present in the soil (Margaluz Arias-Gil, et al. 2007).

The yeast Saccharomyces cerevisiae can use amino acids, ammonia, uracil, purine derivatives and urea as nitrogen sources. The yeast growth rate depends on the nitrogen compounds available in the medium: shorter generation times were recorded in the presence of ammonia, glutamate, asparagine and glutamine (Cooper, 1982).

Nitrogen compounds are incorporated into the cells by means of active transport systems (membrane H1 - ATPases), which involve a general amino acids permease (encoded by GAP1 gene) and other permeases with a variable degree of specificity for particular sets of amino acids (Cooper, 1982; Cartwright et al., 1989; Henschke & Jiranek, 1993; Walker, 1998). Ammonia transport involves the already-known proteins Mep1p, Mep2p and Mep3p (Marini et al., 1994, 1997). Yeast cells are capable of selectively using good nitrogen sources (glutamine and ammonia) in preference to poor ones (proline and urea). This selectivity is accomplished through the nitrogen catabolite repression (NCR), a regulatory mechanism that inhibits expression of the genes required for the uptake and degradation of poor nitrogen sources when cells are provided with a good nitrogen source.

When the good nitrogen source is exhausted, or a poor nitrogen source is provided in its place, NCR is relieved and the expression of NCR-sensitive genes increases (Cooper, 1982; Wiame et al., 1985; Magasanik, 1992; Cooper, 1996). The factors Gln3p and/or Gat1p are responsible for the transcriptional activation of these genes (Mitchell & Magasanik,

1984; Courchesne & Magasanik, 1988; Stanbrough et al., 1995). In the presence of poor nitrogen sources, these activators are released from their interaction with Ure2p in the cytoplasm when good nitrogen sources are available (Beck & Hall, 1999). The expression of genes involved in nitrogen anabolism is also under transcriptional control.
The general amino acid control affecting many biosynthetic pathways is mediated by the Gcn4p transcription factor (Hinnebusch, 2005). Recently, a systematic approach compared the expression levels of 5690 yeast genes in cells growing on 21 alternative nitrogen sources (Godard et al.,2007).

Wine is complex and contains several nitrogen sources. The nature and the availability of nitrogen compounds play a significant role in the wine aroma (Lambrechts & Pretorius, 2000). Hence, the growth of yeast cells in the must can help us understand how nitrogen metabolism is regulated and how the differences in nitrogen availability can affect growth and production of volatile compounds during this process. The major nitrogen compounds in the must are certain amino acids, ammonia, nitrates, proteins, polypeptides, biogenic amines and vitamins (Millery et al., 1986; Monteiro & Bisson, 1992; Aerny, 1996; Feuillat et al., 1998). The assimilation and degradation of these compounds is not equal for all of them and depends on the transport efficiency, the possibility of conversion into ammonia and glutamate without releasing toxic compounds and on the requirements of energy and cofactors (Bisson, 1991). Around 50% of the ammonia and the amino acids arginine, asparagine, aspartate, glutamine, isoleucine, lysine, methionine, serine and threonine are consumed during the first 20 h of vinification (Jiranek et al., 1995). Several studies have allowed a clear understanding of the effects of nitrogen depletion or addition in the global yeast gene expression during alcoholic fermentation (Backhus et al., 2001; Marks et al., 2003; Rossignol et al., 2003; Mendes-Ferreira et al., 2007). However, no comparative analyses of this kind with different nitrogen sources have been published to date. In a recent report, found that the source of nitrogen (ammonia or amino acids) added to a nitrogen-depleted medium during the vinification affects the levels of organoleptic compounds such us 2-phenylethanol, and the expression of several genes involved in nitrogen metabolism (GAD1 and GDH1) and stress response (ERG10) (Elena Jim ´enez-Mart´, et al. 2007).

In grape juice, ammonium is the preferred nitrogen source. As ammonium is consumed, amino acids are taken up in a pattern determined by their concentration relative to cell needs for biosynthesis and to total nitrogen availability (Monteiro, et al. 1992). Two exceptions are known: proline is not taken up from grape juice under anaerobic fermentative conditions (Ingledew W M, et al. 1987) and proline metabolism requires oxygen and a functioning electron transport chain to cleave the proline ring (Wang S S, et al. 1987) and arginine and γ-

aminobutyrate are usually taken up during the latter stages of fermentation under enological conditions and are always detectable in the final wine. Proline and arginine are the most common nitrogenous compounds in grape juice and represent 30 to 65% of the total amino acid content of grape juices (Henschke P A, et al. 1991). Both amino acids require the proline utilization pathway for conversion to glutamate and ammonia (Brandriss M C, et al. 1980). Proline is transported into *S. cerevisiae* by the general amino acid permease and a proline-specific permease (Magasanik B, 1992.). Proline is converted to glutamate in the mitochondria by proline oxidase (product from one gene), (Krzywicki K A, et al. 1984). During alcoholic fermentation, the key role of assimilable nitro-gen is well known. Nitrogen is necessary for protein synthesis and cell growth. It represents an essential nutritional factor for yeasts. Allowing protein synthesis and cell growth, its concentration impacts on fermentation kinetics. Bely et al. (1990) showed that maximal CO2 production rate was highly correlated with assimilable nitrogen concentration. Below 140 mg N/L yeast growth is limited and the fermentation tends to become slow (Agenbach, 1977). Assimilable nitrogen is also important for the synthesis of markers of wine sensory quality such as higher alcohols and esters (Cheynier et al. 2010).

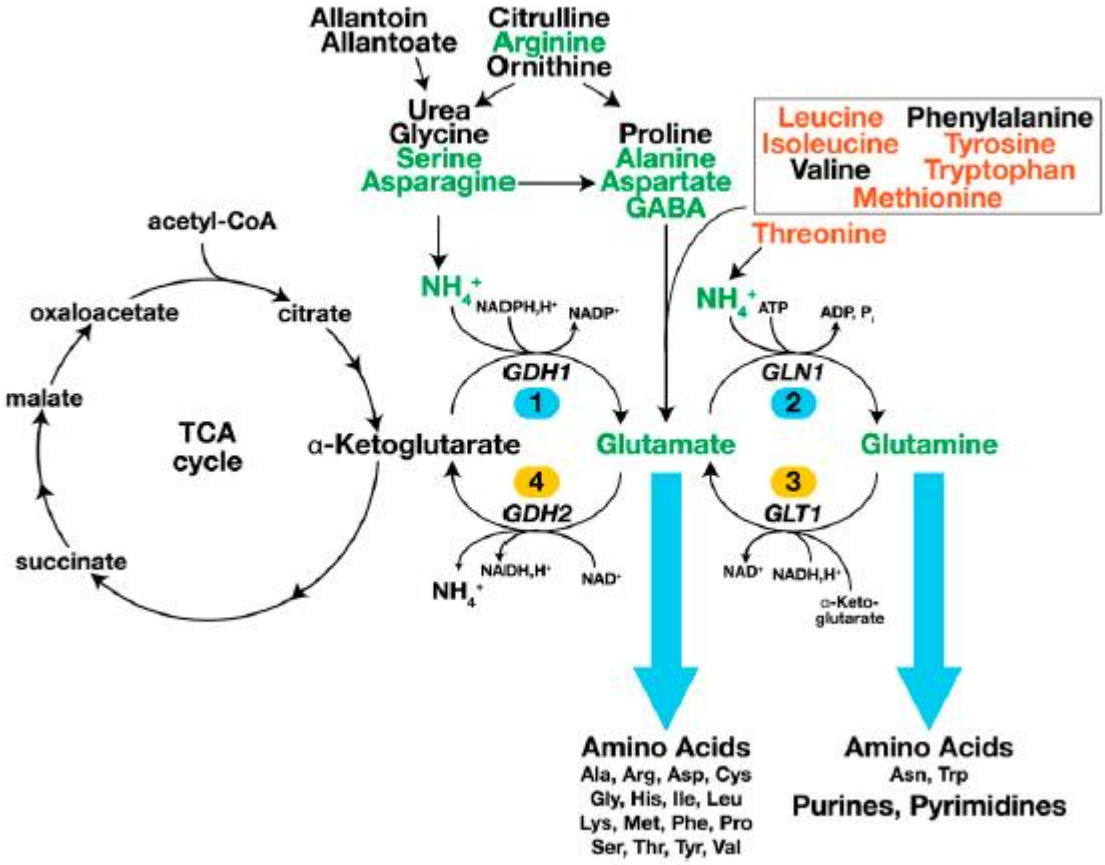


Figure 1 Schematic diagram of the main pathways of nitrogen metabolism. (O. Ljungdahl, et al. 2011)

Yeast cells react to the nitrogen content of the growth environment by controlling nitrogen source uptake and by regulating catabolic and anabolic processes. (Cooper, 1982) and schematically depicted in Figure1**,** the ability to use amino acids and other nitrogenous compounds requires their internalization, and accordingly, yeast cells possess multiple permeases to facilitate their transport across the plasma membrane (O. Ljungdahl, et al. 2011). Notably, the presence of external amino acids induces the expression of several broad-specificity permeases; hence, amino acids induce their own uptake. This transcriptional response is mediated by the plasma membrane localized Ssy1-Ptr3-Ssy5 (SPS) sensor (reviewed in Ljungdahl 2009). Once internalized, nitrogenous compounds can be used directly in biosynthetic processes, be deaminated to generate ammonium, or serve as substrates of transaminases that transfer amino groups to a-ketoglutarate to form glutamate (reviewed in Cooper 1982; Magasanik 1992; Magasanik, et al. 2002). In cells grown on glucose, ammonium can be assimilated by two anabolic reactions, i.e., the synthesis
of glutamate from ammonium and a-ketoglutarate catalyzed by the NADPH-dependent glutamate dehydrogenase (GDH1) (reaction 1) (Figure1), and the synthesis of glutamine from

ammonium and glutamate by glutamine synthetase (GLN1) (reaction 2). In cells grown on ethanol as a carbon source, a Gdh1 isozyme encoded by GDH3 is expressed and contributes to the assimilation of ammonium (Avendano et al. 1997; DeLuna et al. 2001). When glutamine is the sole nitrogen source, the NADH-dependent glutamate synthase (GLT1) is required to catalyze the synthesis of glutamate (reaction 3). The catabolic release of ammonia from glutamate (reaction 4) is catalyzed by the NAD+ linked glutamate dehydrogenase (GDH2). This latter reaction is also required to provide ammonium for the synthesis of glutamine when glutamate is the sole nitrogen source. The central importance of glutamate and glutamine in biosynthesis of nitrogenous compounds is illustrated in Figure
1 (blue arrows); 85% of the total cellular nitrogen is incorporated via the amino nitrogen of glutamate, and the remaining 15% is derived from the amide nitrogen of glutamine (Cooper 1982; O. Ljungdahl, et al. 2011).

2.2 Terpenes:

Terpenes constitute a large family of compounds (about 4,000). Within this family, compounds which are likely to be odorous are monoterpenes (composed of 10 carbon atoms) and sesquiterpenes (composed of 15 carbon atoms), formed respectively from two to three isoprene units. Monoterpenes exist in the form of simple hydrocarbons (limonene, mircene ...), aldehydes (nerals, gernias ...) and esters (geranial acetate ...). (Riberau-Gayon et al., 1975; Marais, 1983; Strauss et al., 1986; Rapp, 1987).

In the grape, about 40 of the most odorous terpene compounds belong to the class of monoterpene alcohols, particularly linalol, α-terpinol, nerol, geraniol, citronellol and OH-trienol, whose smell reminds the essence of rose.

The olfactory perceived thresholds of these compounds are very low, being between about ten and several hundred µg / L; Moreover, their olfactory impact is synergistic and play a more important role in the aroma of the grapes and wines. The most delicious are citronella and linalol. These also play a role in the grapes of Alsatian and German vines: Gewurztraminer, Pinot Grigio, Riesling Rhinos, Auxerrois, Scheurebe, Muller-Thurgau. Monoterpenic alcohols also give a muschè character to Viognier, Albarino and Muscadelle. The monoterpenol content of simple-grape vine wines (Sauvignon, Syrah, Cabernet, Merlot, etc.) is generally lower than the threshold of perception. Monoterpenic and sesquiterpene hydrocarbons with a resinous odor were identified, including limonene, α-terpenine, p-cymene, mircene. Alderides (neral and geranium), acid (transgeric acid), and monoterpenic esters (neril acetate and geranium acetate) were found in grapes and wines (Schreier et al., 1976; Di Stefano, 1993). The development of Botrytis cinerea can also modify the terpenic composition of grapes and their transformation into generally less odorous compounds. For example, 8-OH-linoleum, found in musts and healthy grapes, is also produced by enzyme oxidation of linalol by Botrytis cinerea.

It has been shown that the main monoterpenols and terpenic polyols exist in the grapes in the form of glycosides, in which the constituent sugars are glucose, arabinose, rhamnose and apiose. Grapes contain β-glucosidases capable of releasing certain odorous terpenols from their non-odorous heterosides (Baynove et al., 1984). In the vinification conditions, the incidence of these endogenous enzymes on the development of the aroma of the must is limited by the optimum pH of about 5, due to lack of specificity for certain aglycones and finally for the clarification of the musts which limits their glycosidic activity .

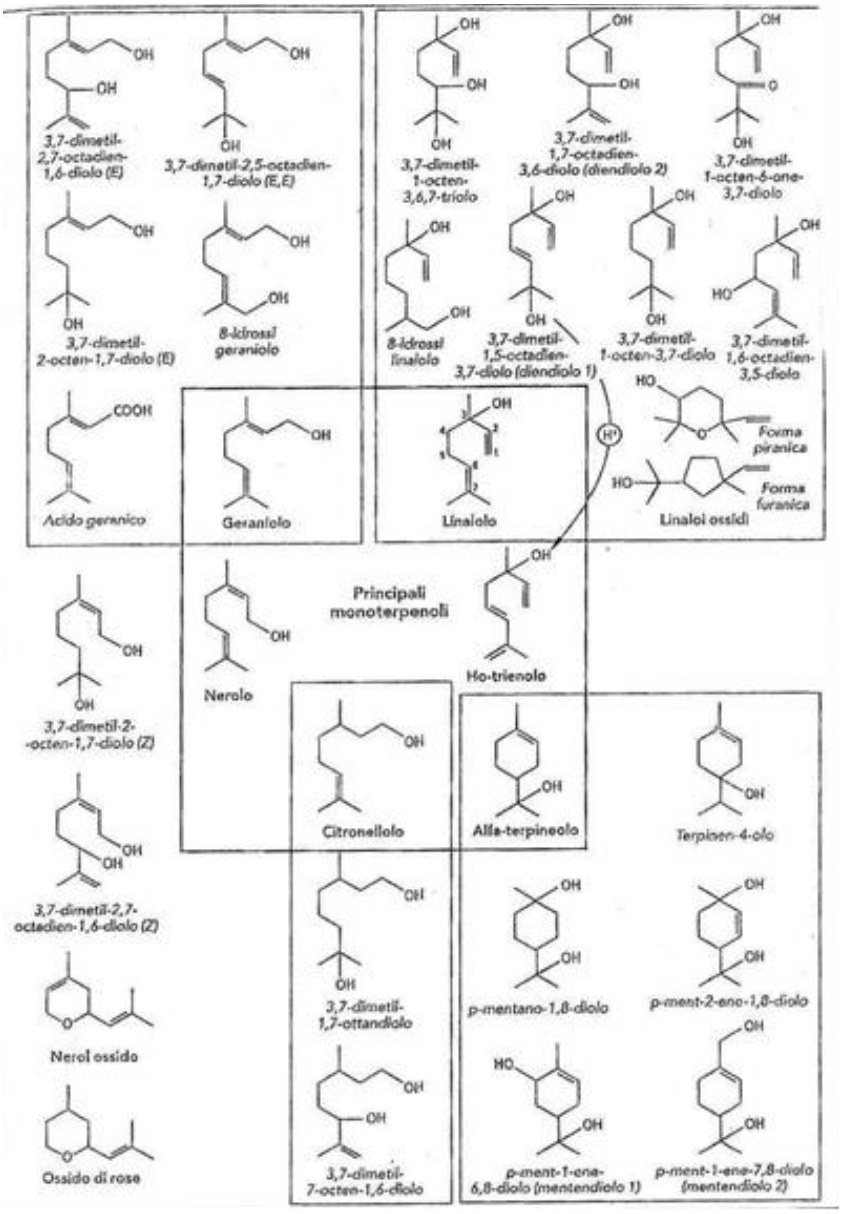


Figure 2: Terpenic compounds (Riberau-Gayon et al., 1975)

2.3 Pyrazines:

Pyrazines are nitroginated ring structures produced as a secondary product of amino acid catabolism in grapes and therefore directly derived (Allen et al., 1991). They are found in stems, grapeskin and seeds, of which the stems contain the highest amount (Roujou de Boubee et al., 2002). Taking a closer look on the grape parts used during white wine vinification, they are located to 67 % in the grapeskins (Ribereau-Gayon et al., 2006). The three MPs most common in wine are; 3-isopropyl-2-methoxypyrazine, IPMP, 3-isobutyl-2-Methoxypyrazine, IBMP, and 3-sec-butyl-2-methoxypyrazine, SBMP (Murray & Withfield, 1975).

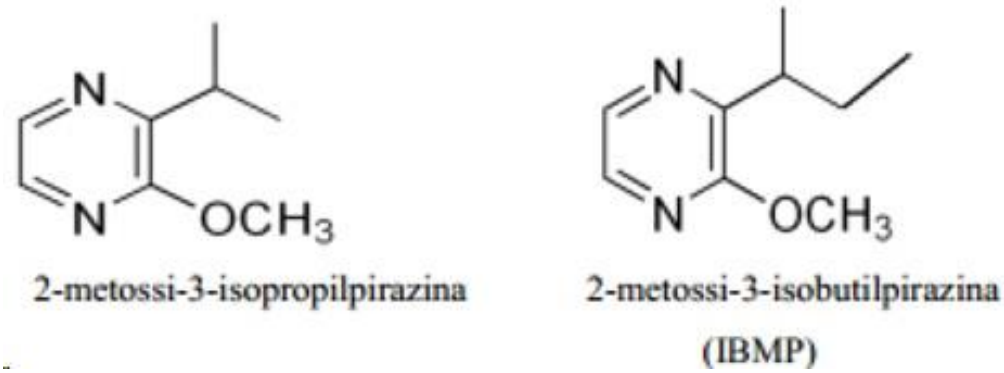


Figure 3: Structural formulas of the three most common Methoxypyrazines.

The main compound contributing to the aroma profile of Sauvignon blanc wines is 2-Methoxy-3-(2-methylpropyl) pyrazine also known as 2-methoxy-3-isobutylpyrazine or 2-isobutyl-3-methoxypyrazine, IBMP (Ribereau-Gayon et al., 2006). It was first detected in Sauvignon blanc wines in 1982 (Augusty et al., 1982). IBMP is detectable at concentrations as low as 2 ng/l but in wines its concentration varies between 12 ng/l and 26 ng/l (Allen et al., 1991). Research of Lacey et al., (1991) detected ranges between 0.6 ng/l and 38 ng/l in wines coming from different origin. IBMP is recognized to bring a greenish, bell pepper aroma, while at lower concentrations, it may only be recognized as a more general vegetal aroma or flavour. Other MP reach much lower values in the final wines. IPMP was reported to be found up to 4.5 ng/l in wines derived from Cabernet Sauvignon and SBMP up to 11.2 ng/l (Sala et al., 2005). It was also suggested, that at higher concentrations, MP may mask other fruity aroma compounds (Chapman et al., 2004).

IBMP was shown to be photodegradable (Heymann et al., 1986) and affected by growing temperature (Lacey et al. 1991). This leads to the conclusion, that viticultural practises affecting canopy architecture and fruit zone microclimate highly affect MP. In fact, higher IBMP levels have to be expected from shaded microclimates. Also higher IBMP concentrations have to be expected from grapes coming from cooler growing regions or years with lower average temperature (Lacey et al., 1991) MP are known to highly decline with ripening. 96 % loss of

the initial IBMP concentration was detected at harvest at maturity stage (Lacey et al. 1991). Similar results were observed by Sala et al., (2005) in Cabernet Sauvignon wines where IBMP concentrations decreased up to 90 % from veraison to harvest. This study revealed similar behaviour other MPs, showing a decrease of 94 % for IPMP and 43 % of SBMP.

Research on Cabernet Sauvignon grapes showed, that an increase in yield would decrease IBMP concentrations in the pressed juices. Therefore, pyrazine concentrations correlate like sugars and anthocyanins inverse with crop yields. Also the intensity ratings of bell pepper aroma, vegetative aroma, and vegetative flavour by mouth were all positively correlated with the IBMP concentration in wines (Chapman et al., 2004).

Sala et al., (2005) showed that MP are correlated with planting density. Denser planted vineyard showed higher amounts of MP. Further the effect of irrigation was studied and irrigated vineyard showed to have higher MP levels.

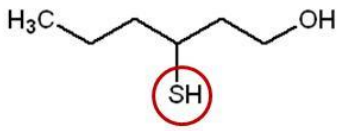
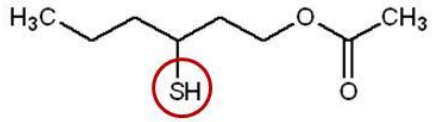
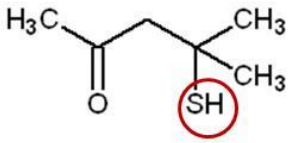
IBMP was less affected by the amount of pressure applied during commercial grape pressing but increased by skin contact (Magu et al., 2007). These results disagree with the findings of Roujou de Boubee et al., (2002) who found that press wines had higher MP levels, which is a more obvious result, as MPs are located to higher amounts in the skin than in the berries flesh.

Kotseridis et al. (2008) showed in a trial with different turbidity levels between 25 and 1280 NTU, that 3-isopropyl-2-methoxypyrazine (IPMP) levels correlate with the amount of turbidity left in a juice before fermentation. The amount of IPMP was positively correlated with the time, juice was prefermentative allowed to settle. The usage of bentonite further increased the effect of settling. This research showed also, that the amount of (IPMP) increased in all turbidity levels during fermentation which was suggested to be a result of grape solid degradation during fermentation.(Roujou de Boubée et al., 2002).

2.4 Thiols:

Sauvignon blanc belongs to the so called "simple flavoured" varieties. This means that musts arising from this variety show no or barely any traces of the typical aromatic characteristics of the grape (Peynaud E., 1980). Higher odours only develop during fermentation (Tominaga et al.1998). The aromatic compounds responsible for that are volatile thiols with additional functional groups as ketones, alcohols and esters (Coetzee, et al., 2012). Among the many thiols found, three volatiles thiols have been identified to have a major influence in the typical aroma of Sauvignon blanc: 4-Mercapto-4-methylpentan-2-one, 4MMP (Darriet et al., 1995), 3-Mercaptohexan-1-ol, 3MH (Tominaga et al. 1998) and 3-Mercaptohexyl acetate, 3MHA (Tominaga et al. 1996).

Table 1: Molecular structures of the species of thiol aroma compounds (Enoviti, 2013)

Compound	Structure	Aroma
3-mercaptohexan-1-ol (3MH)		Passionfruit Grapefruit Gooseberry Guava Box hedge
3-mercaptohexyl acetate (3MHA)		Passionfruit Grapefruit Gooseberry Guava Box hedge
4-mercapto-4-methyl-pentan-2-one (4MMP)		Passionfruit Grapefruit Gooseberry Guava Box hedge

4MMP gives a smell of box tree, passion fruit, broom, black current, citrus zest and grapefruit, but can also give hints of cat urine, if present in higher amounts (Darriet et al.,1995; Dubordieu et al., 2006). 3MH brings odours of passion fruit, grapefruit, gooseberry and guava (Tominaga et al.,1998) and 3MHA reveals passion fruit, grapefruit, box tree, gooseberry and guava (Tominaga et al. 1996). Thiols have very low sensory perception threshold: 4MMP is the lowest, detectable from 0.8 ng/l. The amount of 3MH needed to be detected is 60 ng/l and finally 4 ng/l are required to make 3MHA findable in wines (Dubourdieu et al., 2006).

Although a lot of research has been carried out during the last years in this field, pathways to free volatile precursors from their non-odorous precursors are not indicated completely yet and the context to final thiol concentration in wine still remains unclear. Therefore, this is still a topic of recent research. Especially the formation of 3MH seems to be quite unpredictable as the precursor formation process is dynamic and affected by multiple factors. Three pathways for the formation of 4MMP and 3MH in *saccharomyces cerevisiae* have been identified so far.

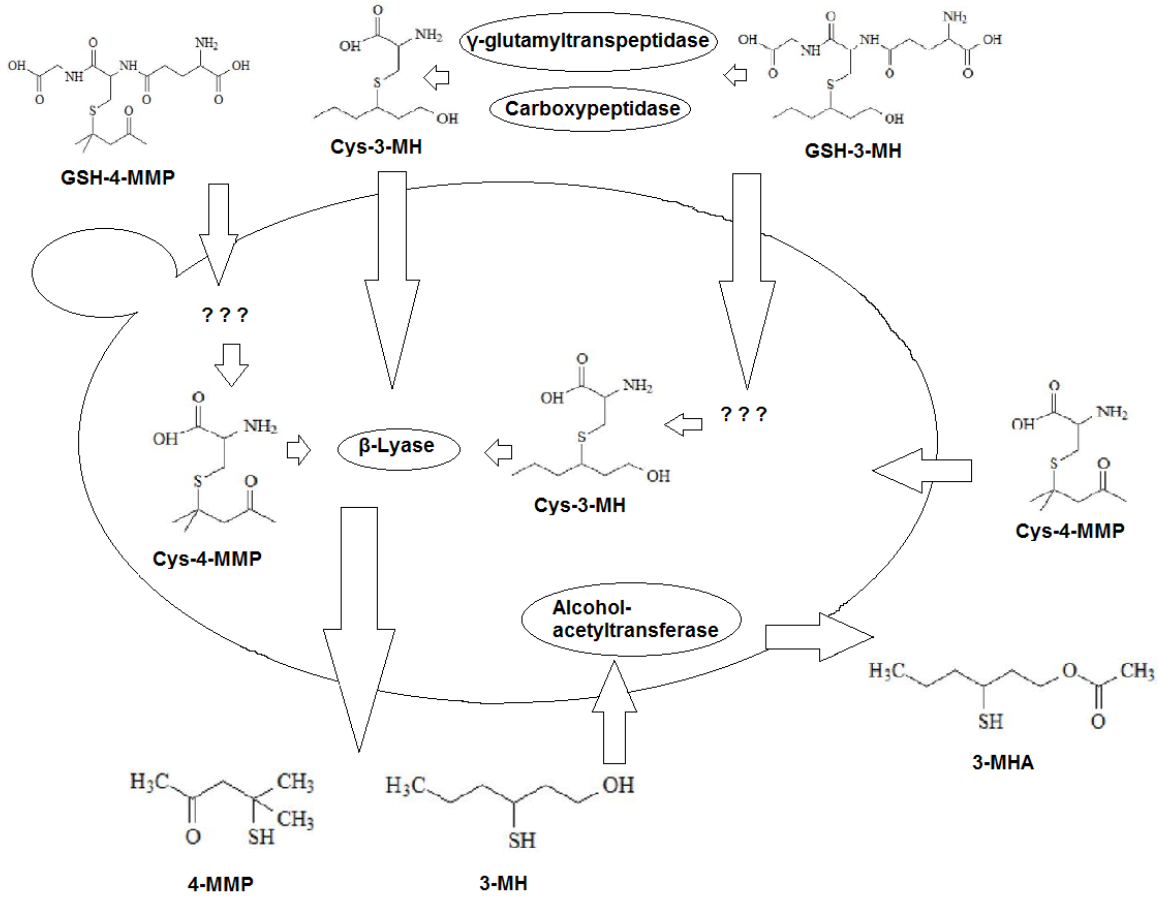


Figure 4: Genesis of the three most common thiols, from their non-odorous precursors

1. The first pathway described, is the direct transfer of the Cysteinlyated precursors: 3-S-cysteinylhexan-1-ol (Cys-3MH) and S-3-(4-mercapto-4-methylpentan-2-one)-cysteine (Cys-4MMP) by beta-lyase (Tominaga et al. 1998).

2. The second pathway describes the presence of S-3-(hexan-1-ol)-L-cysteine in grapes as an intermediate in the breakdown process of S-3-(hexan-1-ol)-glutathione, arising from detoxification processes in vines. Enzymes involved in this process have been suggested to be γ-glutamyltranspeptidase and probably carboxypeptidase first by Gachons et al., (2002). Clear relationship was shown by Roland et al., (2010), using stable isotope dilution assay. This pathway was finally confirmed by recent studies with more sensible methods (Capone et al. 2011).

3. The third pathway is a convertion of (E)-2- hexenal into 3MH and mesityl oxide as a precursor which can be converted to 4MMP. This pathway is proposed to require either the presence of a sulfur donour as H2S, cystein or glutathione to finally form several possible adducts (Schneider et al., 2006, Roland et al., 2010).

(Swiegers et al., 2005) showed that no cysteinlyated precursors exists for 3MHA. This compound is only formed during fermentation by *saccharomyces cerevisiae*. The enzyme shown to be responsible for this transfer is alcohol acetyltransferase, the same enzyme responsible for the formation of the ester ethyl acetate. This study also revealed that the formation of 4MMP does not correlate with the cells ability to form 3MH.

There is a big pool of potential aroma still locked in Sauvignon blanc wines after fermentation, which might be able to be unlocked by winemakers in the future. The amount of 3MH gained from the transformation of Cys3MH represents only 3-12 % of the total amount in wine (Masneuf et al., 2006; Subileau, 2008). Only 10 % of 3MH were shown to arise from green leave volatiles as demonstrated in point 3.) (Schneider et al., 2006).

Recent studies focusing on genes involved in the conversion of cys-4MMP and glut-3MH to their related thiols 4MMP and 3MH showed that the yeast gene IRC7 is essential for this transformation (Thibon et al., 2008). In addition it was shown that the gene OPT1 is required for the uptake of glut-3MH into the yeast cell, although further transporters may be required for this step in some yeast genomes and CIS2/ECM38, encoding γ-glutamyltranspeptidase to further transfer glut-3MH to cys-3MH as part of the detoxification process of electrophilic xenobiotics (Ubiyvovk et al., 2006). The full length IRC7 gene is then not only essential for the release of 3MH but was also found to be the gene responsible for the transformation of cys-4MMP to 4MMP (Santiago & Gardner, 2015). Former research has already revealed the encoding of beta-lyase by IRC7 and have shown a preference of the enzyme towards cys-4MMP to cys-3MH (Roncoroni et al., 2011). Other research also revealed the effect of STR3 to the encoding of beta-lyase, although its effect was found to be rather minor (Harsch & Gardner, 2013). The conversion of cys-3MH to 3MH is only partly affected by the IRC7 gene (Santiago & Gardner, 2015).

The levels of thiols found in bottled Sauvignon blanc wines are strongly influenced by both, viticultural practises as well as winemaking conditions and enological treatments.

It was shown, that precursors greatly increase during ripening, except for the cysteinlyated conjugate of 4MMP, especially in the pre-harvest time (Roland et al., 2010; Capone et al., 2011; Cerreti et al., 2015). Moderate water stress can increase (Cys-3MH) and decrease (Cys-4MMP) concentration in musts, but more severe water stress will lead to a degradation of the final concentration of cysteinlyated precursors (Choné, 2001; Peyrot des Gachons, 2002). To supply the vine with sufficient amount of nitrogen has shown to improve the aromatic potential by increasing cysteinlyated precursor levels (Choné et al., 2006). The foliar application of a combination of sulphur and nitrogen prior to veraison showed to have a volatile thiol expression in wines obtained, without negative impacts on yield and vine vigour. No increase in *botrytis cinerea* was seen in this study (Lacroux et al., 2008).

Besides the many changes occurring by an infection with *botrytis cinerea*, levels of volatile thiols have been shown to increase strongly in wines made from botrytisized grapes both, analytical and sensorial (Sarrazin et al., 2007). Especially S-3-(hexan-1-ol) cysteine (P-3SH) has been shown to be affected strongly by infection with the fungus, due to a stimulation of the grapes metabolic pathway (Thibon et al., 2009).

3MH-S-cys levels were seen to increase when higher pressures were applied during the pressing of grapes. Also skin contact showed an increase of 3MH-S-cys, precursor for varietal aroma (Maggu et al.,2007; Roland et al., 2011). These observations are explainable by the fact that more than 50% of 3MH precursors are found in grapeskins, though 80% of 4MMP precursors are found in the berry flesh (Peyrot des Gachons, 2002). An extraction of polyphenols though was shown to have an indirect negative impact on thiols. Research by Blanchard et al. (2004) revealed higher oxidation of 3MH in the presence of oxygen and catechin compared to the presence of only oxygen. It was shown that must oxidation affected hydrocinnamic acids which form quinones and usually later react with glutathione via a Michael addition, to GRP (grape reaction product) due to the presence of phenoloxidase (Singleton et al., 1985; Singleton, 1987). But quinones are also able to directly react with thiols within a Michael addition or form peroxides as a result of multiple reactions (Cheynier et al., 1986; Nikolantonaki et al., 2012). Showed also that thiols react differently with quinones, 4MMP was shown to be less affected than 3MH. The thiol-oxidative capacities of peroxides have more recently been confirmed (Blanchard et al.,2004). Must oxidation still seem to be a way to increase G3MH and Cysteinlyated precursors and G4MMP are not decreased, as their sulfhydryl group is involved in a C-S bond which preserves them from oxidation (Roland et al., 2010). These results have to be rated with caution though as they may only match wines with a very low polyphenol fraction. No effect has been seen on different pH and potassium levels in Sauvignon blanc juice (Grose et al. 2015). But a higher oxidation of polyphenols is known to occur at higher pH, which ends in an indirect negative effect on thiols as described above. The use of SO2 is known to prevent phenol oxidation by inhibition of phenoloxidases and is therefore an important factor of conserving thiol oxidation.

Saccharomyces cerevisiae offers different abilities to free volatile thiols from their non-odorous precursors, especially in their aptitude to release 4MMP from its cysteinlyated precursors. Research by Howell et al., (2004) revealed abilities of certain yeast strains to release 4MMP from its cysteinlyated precursor up to 138 fold in synthetic medium, compared to a control strain. It was shown that the release of 3MH is not affected by the yeast strain. The most efficient commercial wine yeast can only release approximately 5% from precursors (Murat et al., 2001; Swiegers et al. 2006). In another study, a transfer rate between 0.6% and 10.2% with an average of 3,2% was shown in rose wines coming from AOC Bordeaux (Murat et al.,2001b). Studies have also shown that some non-*saccharomyces* strains may contribute to

the final volatile aroma concentrations found in wines, especially to the amount of 3MH released (Zott et al.,2011).

Masneuf-Pomaréde et al., (2006) clearly demonstrated the effect of fermentation temperature on volatile thiol transfer. 4MMP as well as 3MH were found to be higher when fermented at 20°C, compared with levels achieved at 13°C fermentation temperature. In contrast Swiegers et al., (2006) found that a wine fermented at 18°C had higher amounts of 4MMP than wines fermented at 23°C and 28°C. Studies of Howell et al. (2004) on the effect of fermentation temperature on the abilities of different yeast strain to release 4MMP have shown that temperature was an increasing factor for some strains but not for all, leaving the conclusion that other factors need to be considered to qualify the total effect of temperature.

Former research has proven the positive effect of low fermentation temperatures (10°C) on fruity esters as isoamyl acetate, isobutyl acetate, ethyl butyrate, hexyl acetate compared to the formation of more "heady" esters as ethyl octanoate, 2-phenethyl acetate and ethyl decanoate at higher temperatures during fermentation at 15 - 20 °C (Killian & Ough, 1979). Additionally, the research conducted by Masneuf-Pomaréde et al., (2006) also showed an increase in acetic acid in wines, fermented at higher temperatures. More research has to be carried out to find perfect fermentation temperatures at which wines would profit from both, fruity esters and volatile thiols, leading to a maximisation of total wine aroma.

Subileleau et al., (2008) showed that the addition of diammonium phosphate decreases 3MH production. This is suggested to be an effect of the yeast cells nitrogen catabolism repressing GAP1, an identified transporter molecule, responsible to transport Cys-3MH into the cell. This suppression can be extended under nitrogen rich conditions. These findings have also been confirmed more recently by Harsch et al. (2013) who found that thiol yields are not only modified by nitrogen containing but also sulphur containing supplements. Among 17 genes involved in sulphur amino metabolism, MET17, CYS4, and CYS3 were standing out, responsible for the multiple step conversion of H2S to cysteine. Nitrogen wise, addition of ammonia, known to induce nitrogen catabolic repression and urea increased thiol production substantially. These results disagree with the findings of Subileau et al., (2008), who found that only addition of Urea had a positive effect on thiols whereas addition of DAP decreased Thiol expression. Taillandier et al., (2007) showed different behaviour towards nitrogen, when monitoring four yeast strains. Therefore, effects of nitrogen content and source have to be related to a specific strain. The addition of nitrogen sources has on the other hand shown to have an effect on the final ester profile of wines, as addition of ammonia increases concentrations of acetate esters and ethyl butyrate but not the higher molecular weight fatty acid ethyl esters. Differences depend on the yeast strain added and source of nitrogen used. A general increase on esters by ammonia addition could not have been proven (Miller et al., 2007). Also Pinu et al., (2013) have monitored changes in ester profile due to the addition of

nitrogen sources. Further an increase of negative perceived aroma compounds as methional (sweet soup and meat aroma) and 4-ethylguaiacol (glove spicy, smoky aroma) could be monitored.

Recently, the presence of the two precursor Cys-3MH and GSH-3MH was proven in commercial, grape derived tannin (Larcher et al., 2013). Tannins are widely known to winemakers for their abilities to stabilize colour in red wine making and to increase mouthfeel and aroma profile. The positive potential of this thiol source as a contribution to the total precursor pool, leading to higher 3MH and 3MHA values, has been shown in recent research (Larcher et al., 2015). Although more research has to be carried out about the perfect usage of this new precursor source, an alternative, introduced precursor source was revealed within this research.

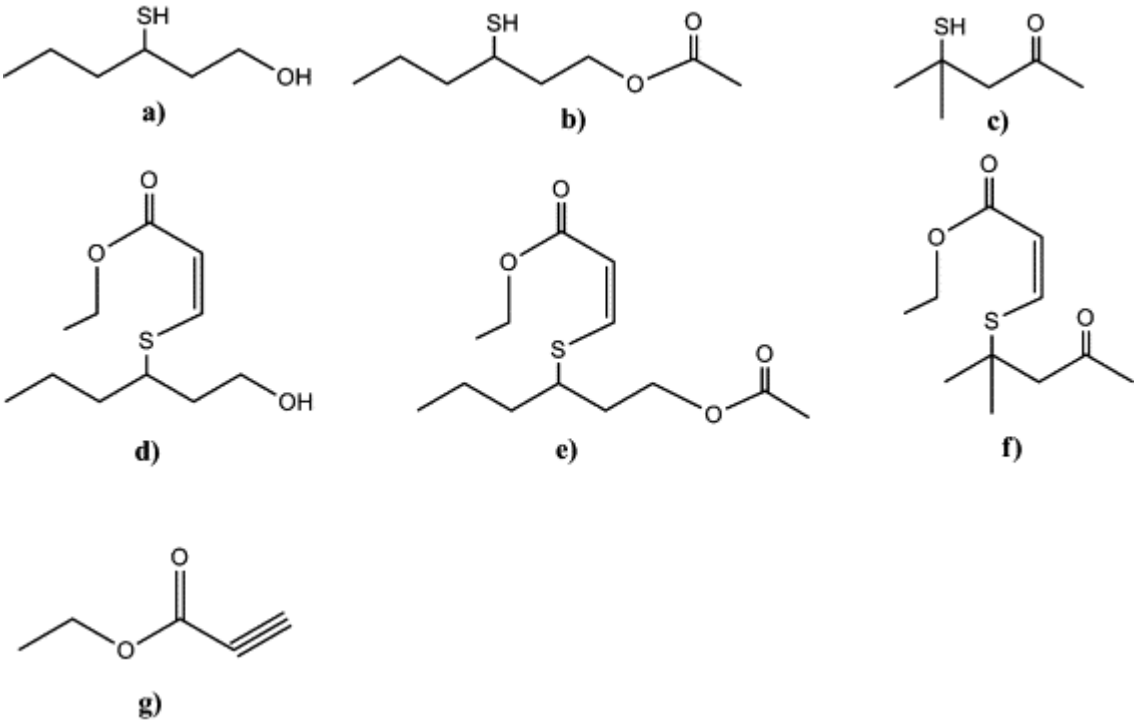


Figure 5. Molecular structures of the species studied. (a) 3-Mercaptohexan-1-ol (3MH); (b) 3-mercaptohexyl acetate (3MHA); (c) 4-mercapto-4-methylpentan-2-one (4MMP); (d) 3-((1'-hydroxyhexan-3'-yl)thio)acrylate (3MH-ETP); (e) 3-((1'-acetoxyhexan-3'-yl)th

2.5 Sterols and Fatty Acids

Winemaking techniques as used in countries considered as "new world" wine regions nowadays are known for a highly reductive style of winemaking when it comes to the production of Sauvignon blanc wines. Modern techniques as stainless steel vats, the use of inert gases, sparging, and the use of antioxidatives are used to prevent oxidase activity and the loss of highly reactive thiols, which lead to the appreciated styles of wines coming from "new world" origins. These techniques can lead to juices with an extreme prefermentative prevention of oxygen. Additionally, prefermentative clarification is applied after pressing. Both techniques may cause troubles during the process of winemaking. Already early in the history

of wine related research, growth rates of yeast in an oxygen depleted medium were described as very low. Further, the supplementation of sterols was first described to significantly increase yeast growth under such anaerobic conditions (Andreasen & Stier, 1953). More recently the sterol needs of yeast were monitored more precisely, specifying the sterol requirement for optimal yeast growth from 2 to 4 mg phytosterols/l (Deytieux et al. 2005).

The cell wall of yeast consists mainly of fluidity providing phospholipids (principally phosphatidylcholine and phosphatidylethanolamine) and rigidity providing sterols, principally ergosterol and zymosterol (Walker G.M., 1998). Therefore, lipids and sterols are key factors of the growth, metabolism, and viability of yeastcells during the alcoholic fermentation.

Yeast cells can synthesize their own sterol, ergosterol, in the membrane of the endoplasmatic reticulum by squalene cyclization, from where it is transported through the Golgi bodies to the plasma membrane in aerobic conditions (Zinser et al., 1991). The requirements of oxygen in a lipid depleted must were estimated at about 10 mg/l to maintain a vital yeast population (Sablayrolles & Barre, 1986.) The enzyme responsible for the regulation of sterol synthesis is hydroxymethylglutaryl-CoA reductase. The activity of this enzyme is highly oxygen dependent. Therefore, yeast becomes auxotroph to sterols and long chained fatty acids under anaerobic conditions (Jacquier & Schneiter, 2012). Besides that, UFAs, especially linolenic acid have been shown to accelerate sterol synthesis up to 7fold. Therefore, it was suggested, that the supplementation of UFAs leads to a completion of the protein synthesizing apparatus during cell growth (Boll et al., 1980).

Only under anaerobic conditions, yeast is able to import exogenous sterols, arising from sources other than fungal (Zavrel et al., 2013). It was shown that the highest UFA uptake rates are observed in the exponential phase of yeast growth, ending in an almost complete consumption (Duan et al., 2015). Luparia et al. (2004) pointed out the importance of solids for nutritional purposes, mainly for their content of grape phytosterols. Casalta et al., (2016) analysed solids from white and red musts and found phytosterol concentrations to range from 3 to 10 mg cholesterol equivalent per gram dry weight. Their composition was described as follows: 89% β-sitosterol, 6% campesterol, 3% stigmasterol, and 3% stigmastanol.

Betrand et al., (1984) have shown that clarification reduces the total fatty acid content in juice to 90%. A supplementation of UFAs on the other hand showed to improved yeast growth and fermentation activity (Duan et al., 2015).

Further, the ability of yeast to produce and tolerate increasing ethanol content during fermentation is highly linked to the amount of unsaturated fatty acids and sterols in their plasma membranes (Rose, 1993)**.**

Saccharomyces cerevisiae was shown to increase UFAs/SFAs and ergosterol content at the expense of the steryl ester pool in in their cell membranes as a way to adapt to stressful conditions during fermentation (Rupčić, et al., 2010). Finally, Tesnière et al., (2013) showed

that lipid limitation affects nitrogen metabolism. A rapid loss of cell viability and higher cell death rates were recorded in a lipid depleted medium, and correlated to higher nitrogen availability. These results implement a possible negative role of nitrogen during fermentation by modulating stress responses. Higher lipid contents were suggested in high nitrogen ferments.

Smith et al., (1996) have shown that ergosterol is not only incorporated by the yeast as part of the cell membrane, but also has effects on gene expression and enzymatic activity.

Research by Duan et al., (2015) clearly showed the positive effect of unsaturated fatty acids (UFAs) on most volatile compounds. The amount of higher alcohols (2-phenylethanol, 2-methyl-1-propanol and 3-(methylthio)-1-propanol), medium-chain fatty acids (butanoic acid, hexanoic acid and octanoic acid), acetate esters (isoamyl acetate and 2-phenylethyl acetate) and all ethyl esters was increased after the addition of UFAs to a synthetic medium. As mentioned before, the enzyme responsible for the formation of ethyl esters, alcohol acetyltransferase is also responsible for the formation of 3MHA from 3MH it is expectable that an increase of UFAs could increase the formation of this compound. To my best knowledge, no research about the effect of UFAs on 3MHA formation was done to date. Pinu et al., (2013) suggested that the initial biosynthesis of 3MH might be influenced by the activity of the TCA cycle and fatty acid metabolism of yeasts as linoleic acid has been shown to correlate with 3MH even if no correlation was found in this study with 3MHA or its acetylation ratio. *Saccharomyces cerevisiae* lacks of Δ12-fatty acid desaturase and ω3-fatty acid desaturase, the enzymes required for the production of polyunsaturated fatty acids as linoleic acid and linolenic acid (Yazawa et al.,2009). Research has revealed the contribution of juice lees to content in linoleic and linolenic acids and related enzymes (Nicolini et al. 2011; Varela et al. 1999). The contribution of PUFAs to cell integrity and a wide stress tolerance is widely known (Kajiwara et al. 1996; Rodriguez-Vargas et al. 2007; Yazawa et al., 2009).

The formation of hexanal and hexenals as (Z)-3-hexenal and (E)-2-hexenal due to the enyzmatic breakdown of linoleic acid was formerly described by Drawert F. (1974). The pathway to form n-hexanal from linoleic acid was described by Matsui, (2006) as a result of lipoxigenase (LOX) being able to oxidise the carbon double bonds of linolenic acid, forming linolenic acid 13-hydroperoxide (13HPOT). Further 13-hydroperoxide lyase (13HPL) is able to form (Z)-3-hexenal and 12-oxo-(Z)-9-dodecenoic acid. LOX is known to be able to break doublebonds at position 9, preferring linoleic acid (100 % activity) over linolenic (60.4 % activity) and oleic acids (46 % activity). It was further shown that the highest activity of this enzyme occurs between 25 and 30°C and an increased activity was monitored in presence of magnesium ions (Busquets et al., 2004). Drawert F. (1974), described these enzymatic-oxidative processes occurring after the crushing of fruit, if oxygen had access to the substrate and if enzymes had not been inhibited previously and the formation of these compounds

leading to grassy, green impression of freshly crushed unripe grapes and leaves. This effect was also described by Roufet et al. (1987) who described the proceeding decrease of linolenic acid during maturation is the reason, that green, unripe, unpleasant flavours are not to be found in juices pressed from ripe fruit. Harsch et al. (2013) proved that a sulphur donor as H_2S is required to further transfer (E)-2-Hexenal and its alcohol (E)-2-Hexen-1-ol into 3MH and 3 MHA. It was also shown that E)-2-Hexenal and (E)-2-Hexen-1-ol are metabolized in the first 24 hours after yeast inoculation, prior to alcoholic fermentation, probably to decrease its fungicide effect, which was mentioned by Kubo et al., (2003).

At the other hand it was indicated that linoleic and linolenic acid influences ester and medium chain fatty acid excretion negatively when fatty acids in the yeast reached a defined concentration. The same study revealed an increase in fusel alcohols when linoleic and linolenic acid was increased (Rosi, et al., 1992).

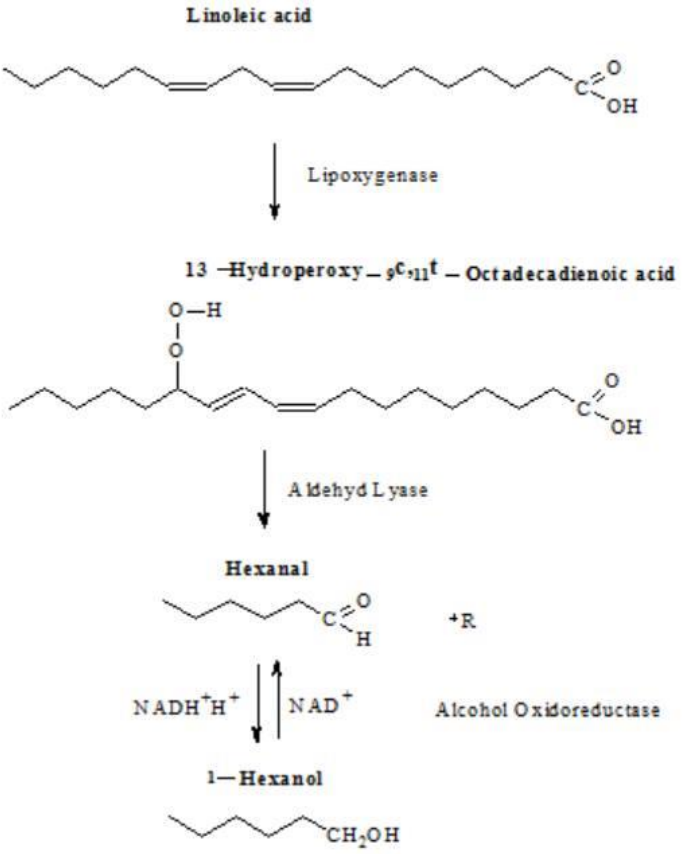


Figure 6: Genesis of hexanol due to oxidation of Linoleic acid. Described by Drawert (1974).

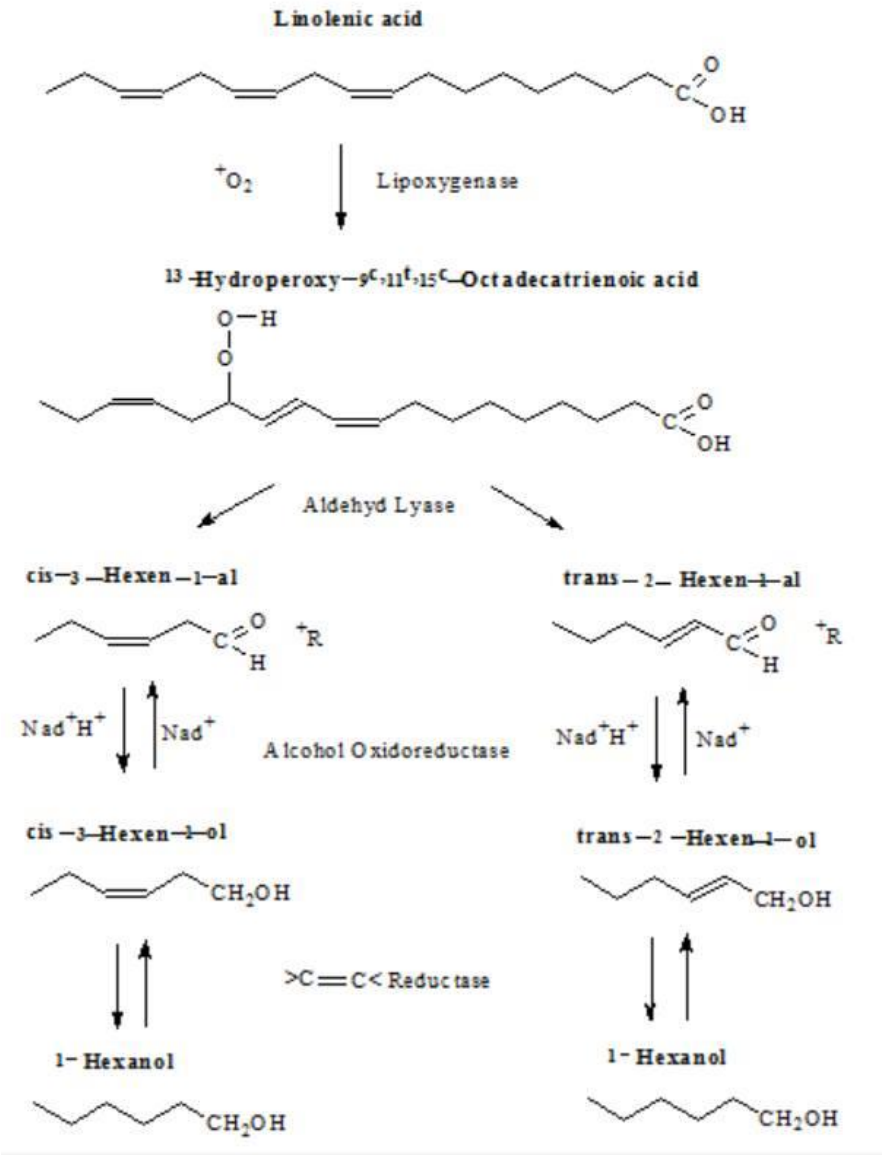


Figure 7: Genesis of hexanol due to oxidation of Linolenic acid. Described by Drawert (1974).

Miller et al., (2007) have also shown that the addition of nitrogen sources, preferable NH_4 but also the addition of amino acids, increased fermentation capacities of yeast and made fermentations finish in a shorter time than control wines. Interesting in the research of Kontkanem et al. (2004) where yeast cells were shown to double more often at lower inoculation rates before going into a stationary phase. This result can be confirmed by the work of Lee et al., (2004) as well. Here, even though initial inoculation rates were different by a factor of two, the average total cell count in between the treatments was less than 15 %. There are two major factors, limiting cell growth, being partly able to supplement each other: one of it is molecular oxygen, partly replaceable by fatty acids and sterol, which objective has been discussed before (Monk, 1997). This leads to the suggestion that yeast, inoculated at lower rates have to struggle more for the limited amount of these nutrients to double till the sufficient cell number. This stress will also have effects on metabolism and gene expression on some

enzymes playing a role in aroma composition and maybe alcohol yield. Dried yeast cell cultures are known to have similar sterol reserves. This implements that a higher inoculation rate might have similar effects as the supplementation with fatty acids and sterols as cells can benefit of their lipid storages and finally don't have to divide that often to reach desirable cell numbers, which is known to dissolve lipid reserves between yeast generations (Deytieux et al. 2005).
If CO_2 would not be released from a ferment it will in the worst case denaturise proteins and enzymes within the yeast cell. It is therefore likely that a higher release of CO_2 may protect enzymes and therefore higher thiols expression may occur (Spilimbergo et al., 2005).
Smith et al., (1996) reported on the effect of UFAs not only on cell wall composition but also on enzyme activity. Swiegers et al., (2005) reported alcohol acetyltransferase, the same enzyme responsible for the formation of the ester ethyl acetate to be responsible for the transformation of 3MH to 3MHA. UFAs are known to increase acetyltransferase activity (Duan et al., 2015). At the same time, most 3MH precursors are found in skin (Peyrot des Gachons, 2002). As turbidity consists mainly of skin debris, an increase of 3MH during fermentation at higher turbidities is expectable. Therefore, the effect of grape solids on thiol production during fermentation should be clarified. Further, interactions of nitrogen compounds on thiol production have been stated (Subileleau et al., 2008). But differences have been monitored between nitrogen sources and yeast strain, effects of different nitrogen sources in combination with turbidity shall be analysed more in advance as suggested by Tesnière et al., (2013). With add different amino acid this means different sources of nitrogen for the yeast, and understand who prefer and what takes first
Yeast influence wine aroma by the following mechanisms: first the biocontrol of molds by yeasts before harvest—mainly by apiculate yeast species competing for nutrients, second the alcoholic fermentation of the grape juice into wine, third the de novo biosynthesis of flavour and aroma compounds during alcoholic fermentation, fourth the metabolism of flavour-neutral grape compounds into active aroma and flavour compounds, fifth post-fermentation impact on wine via autolysis, and sixth the influence of the growth of malolactic and spoilage bacteria. In the figure 3 the biosynthesis of flavour and aroma compounds. (Gustav Styger •et al. 2011)

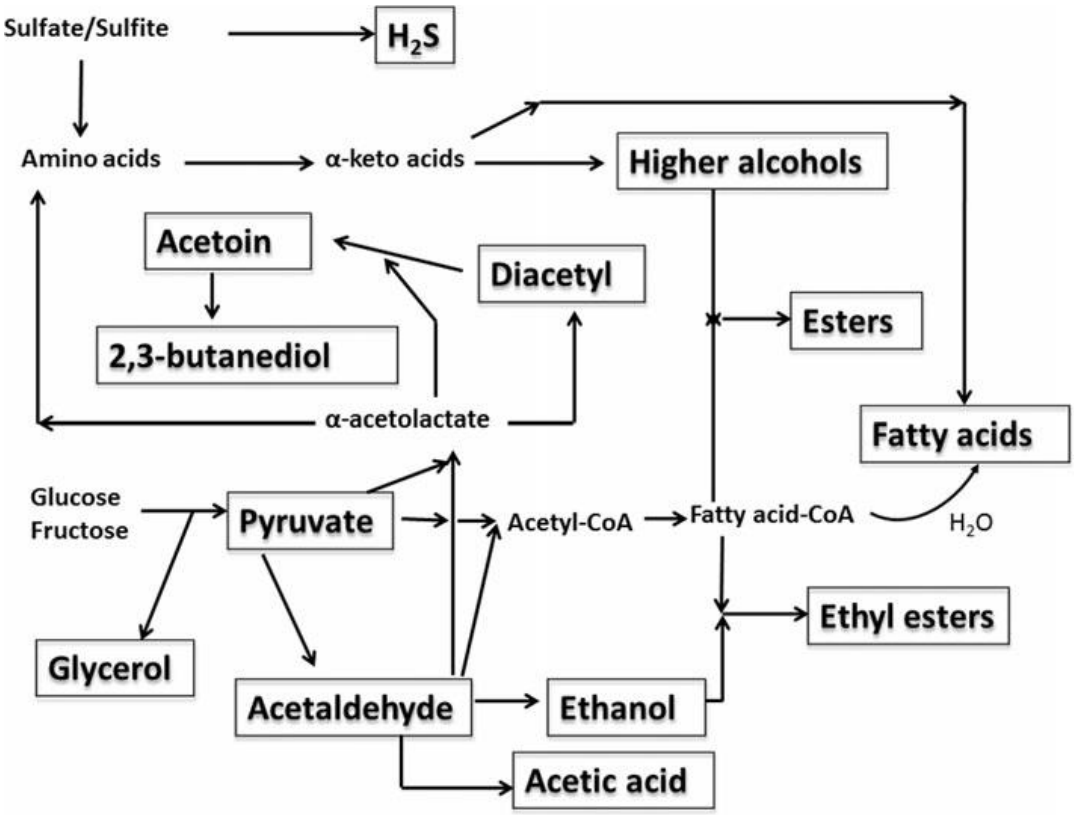


Figure 8: Some of the major classes of aroma compounds (shown in blocks) produced by yeast during alcoholic fermentation as adapted from Bartowsky et al., 2004 and Lambrechts et al,. 2000.

3. Goals of this work:

The main objective of this study was to understand from 10 different source of nitrogen, such as L-Glutamine, L-Aspartic Acid, L-Glutamic acid, Serine, Alanine, L-Arginine, DAP, L-Glutathione reduced, two different BLEND; to understand who prefer the yeast and what takes first, observing during alcoholic fermentation who source is fast or slow for the yeast and looking the amino acid profile before-after alcoholic fermentation to see the quantity of single amino acid and but not just all this also aroma compounds and all analytical analysis in the wine, who have the best way to production the aroma compounds.

4. Materials and Method

4.1 Gifford's Creek Lane Vineyard, block "NGLFSBC"

The Constellation managed but privately owned 8.3-ha vineyard block is comprised of 14-year-old Sauvignon blanc MS clone vines grafted on SO4 rootstock.

Row and vine spacings of 2.4 m x 2.0 m are used on this "moderate to high vigour" site. The soil type is described as a Wairau Mottled Phase – moderately well-drained to well-drained loamy and sandy alluvium soil. Commercial cane loading per vine alternates between three and four canes that are laid to fill the fruiting wires, as opposed to a set node number per vine. The block yield ranges typically from 11–16 T/ha. Irrigation is usually set at 5 L per vine every second day from the first week of November. Nutrition is via fertigation with an annual application of 6.2 kg N/ha in the form of calcium nitrate. Seasonal canopy management typically involves canopy trimming (sides and top) three times during the growing season and fruit zone leaf plucking twice.

4.2 Omaka Vineyard

The vineyard was planted with mass-selected (UCD1) Sauvignon blanc on a SO4 rootstock. All vines are VSP (vertical shoot position pruned) and trellised at 1.8 m between the vines and 2.4 m between the vine rows. Although the soil for the whole vineyard was classified as Wairau silt loam, strata of different soil textures that run in an east-west direction created rapidly changing soil profiles while moving north-south. The vineyard rows were planted along the N-S axis and therefore, along the vine rows, great differences in soil texture horizons and hence soil WHC(water holding capacity) were found: The soils at the northern end of the vineyard were deep silt loams that are classified in the Land Resource Information System Spatial Data Layers (Newsome et al. 2008) as 'non-gravelly to very slightly gravelly', showing high WHC while the soil at the southern end of the vineyard was sandy and classified as 'moderately to very gravelly' and therefore low WHC. The soils in the centre of the vineyard showed intermediate WHC, with smaller stones but are not separately identified in the aforementioned data layers. Work done in Marlborough (Bramley et al. 2011; Trought et al. 2008), found a close relationship between soil class and vine trunk circumference

4.3 Juice

Grape, Sauvignon blanc, two different vineyards from Omaka vineyard and Gifford Creek vineyard, the first harvest start with Omaka Vineyard vineyard 6th April and 11th April Gifford Creek vineyard, pick the grapes all by hand and put in the picking crate for a maximum of

fifteen kilograms, when the grape come in the winery control oxidation, with use the dry ice and inert gas during all process.

Process: the first step is weight the grape from picking crate, next thing destemming and crushing (Eno 1S, Italy) the grape with addition dry ice in crush machine, after that is weighed the juice in the balance with the respective calculation is added 0.8 ml/kg potassium metabisulphite and 0.5 ml/kg enzyme, and then had to stay in cellar fridge for 60 minutes before pressing. Once have done that, insert the must in the hydro press (Marchisio, Italy) start with 2 bar for 3 minutes and 14 minutes 4 bar with addition dry ice, following weighing for add 0.5 g/l of bentonite. The juice after press stay in cellar at 6 ºC close in the stainless-steel tanks with addition argon and wait 24 hours.

Next thing, racking the juice normal winemaking practice, a juice sample was collected for analysis. A final volume is 25 litre for each vineyard of settled juice and add in the 66 bottle 750 ml 33 for each vineyard. Control the turbidity the juice have to start for all bottle with the same turbidity about 150 NTU (nephelometric turbidity unit) added turbicel® (Laffort), the quantity change based on the starting turbidity.

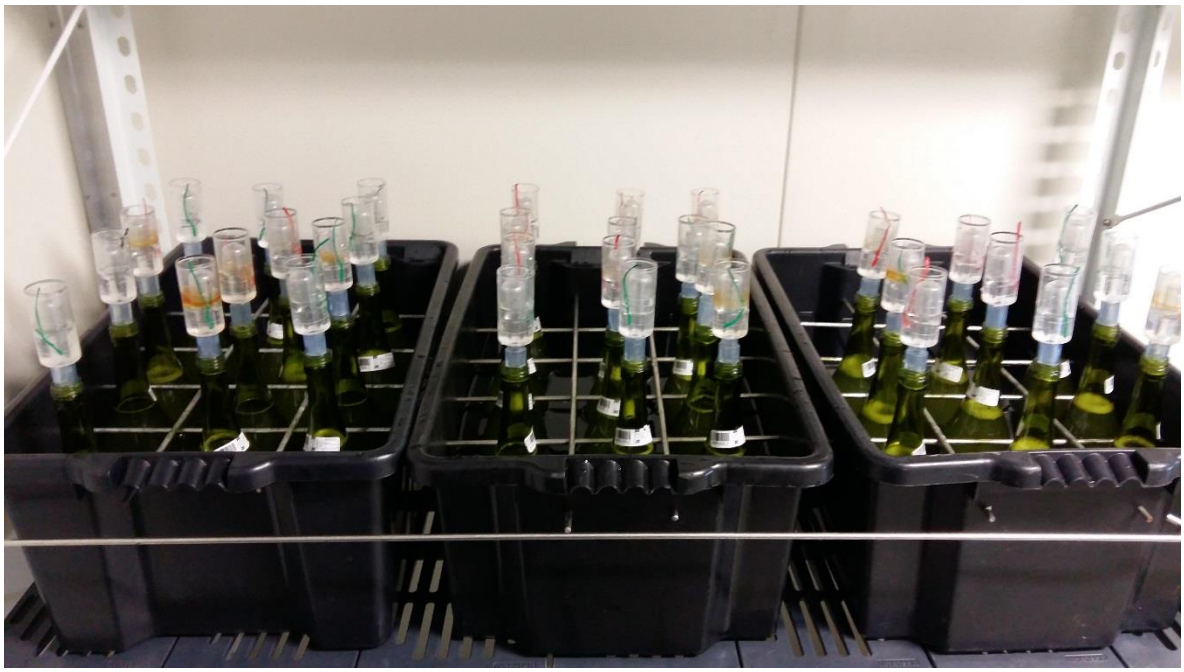


Figure 9 Sample 66 bottle for 750 ml Omaka and Gifford Vineyard

4.4 Winemaking

In general, the physical process of winemaking involves the crushing and pressing of grape berries to obtain grape juice which is then introduced to yeasts (inoculated or un-inoculated) to commence fermentation following the fermentation process the resulting wine is separated from the settled yeast cells (lees). The wine is then temperature stabilized and refined of any undesirable solids and proteins. After this point the wine is filtered and blended before being bottled for consumption (Fleet, 2003; Jackson, 2000).

The protocol of winemaking is:

1. Hand harvest 40kg fruit for each treatment from replicated vineyard plots. No field additions of sulphur.
2. Transfer to winery, crush and destem. Add PMS at 80g/T (40ppm SO2).
3. Add enzyme (50ml/tonne Rapidase Clear (liquid)) to crushed/destemmed grapes.
Enzyme addition: 0.5mls of 10% enzyme solution/kg.
4. Transfer to 25L containers under CO2 cover. Give 1 hour skin contact time at 6˚C under CO2 cover.
5. Transfer to hydro press. Collect free run under CO2 cover. Press off under CO2 cover. Pressing regime:
i. Increase pressure slowly to 2.0 bar, hold for 3 min.
ii. Increase pressure slowly to 4 bar, hold for 14 min.
6. Add 0.5g/L of bentonite slurry to pressed juice.
7. Cold settle juice for 24 hours at 6°C.
8. Rack off solids and transfer juice to 8-10L fermentation vessels.
9. Take 2 x microtubes and 1 x 50ml juice samples and freeze. 1 x 50ml falcon tube for primary juice analysis.
10. Warm juice to 15˚C and inoculate with X5 yeast (250mg/L), rehydrate yeast using standard procedure.

5. General Procedure

5.1 Juice Turbidity:

Turbidity measurements were carried out, using a “Hach-2100Q IS” turbidity meter. Juice turbidity start with Omaka Vineyard with 44 NTU and Gifford Creek Vineyard with 13.4 NTU. Our target juice turbidity is about 150 NTU. Therefore add “Laffort- Turbicel”, purified cellulose ($C_6H_{10}O_5$) to bring the juice to 150 NTU. Procedure: put 5 g turbicel in 250 ml juice, for OV add 13.8 ml and for GC add 17.7 ml of this solution.

5.2 Yeast inoculation:

All sample were inoculated with “Laffort-Zymaflore X5” commercial yeast, known for its thiol releasing properties. Standard procedure of inoculation at PFR is a standard yeast addition of 250 mg/l added to all 750mL. Yeast was rehydrated in water at 35-degree temperature for about 17 minutes, after that 5 minutes at temperature juice and then inoculated by pipette.

5.3 Amino acid addition:

The samples previously prepared were added single amino acids, knowing the respective % of N:

- L-Aspartic acid 10.5% of N, (Sigma – Alorich, $C_4H_7NO_4$)
- Serine 13.3% of N, (Spectrum, $C_3H_7NO_3$)
- L-Glutamine 19.2% of N, (Spectrum, $C_5H_{10}N_2O_3$)
- L-Arginine 32.1% of N, (Arginine, Wikipedia, 2017)
- Alanine 15.7% of N, (Spectrum, $C_3H_7NO_2$)
- L-Glutamic acid 9.5% of N, (Sigma – Alorich, $C_5H_9NO_4$)
- DAP 21% of N, (Nabta, Diammonium phosphate, $(NH_4)_2HPO_4$)
- Glutathione 13.7% of N (Sigma – Alorich – $C_{10}H_{17}N_3O_6S$)

Table 2 Initial nitrogen status in juice OV

NH4+ (mg/L of N)	PAA (mg/L of N)	YAN (mg/L of N)
15.79	**86.19**	**101.98**

For OV considered plus 121.3 mg/L of N to add, the purpose is to have the same amount of nitrogen for the single amino acid

Table 3 Quantity to add amino acid in OV, plus table BLEND1(*) and BLEND2(**)

OV	Treatment	mg/750ml
	CTR	-
	Asp	864.9
	Ser	682.9
	Gln	474.9
	Arg	283.0
	Ala	578.9
	Glu	956.1
	GSH	665.7
	DAP	1.43 ml of 30% of solution
	BLEND1	*
	BLEND2	**

BLEND AA 1*	mg/750ml
L-Aspartic Acid	60.5
Serine	47.8
L-Glutamine	76.0
L-Arginine	90.6
Alanine	121.6
L-Glutamic Acid	162.5

BLEND AA 2**	mg/750ml
L-Aspartic Acid	48.4
Serine	38.2
L-Glutamine	60.8
L-Arginine	72.4
Alanine	97.3
L-Glutamic Acid	130.0
Glutathione	133.1

And then second vineyard Gifford's Creek (high YAN), consider the same method said up, but different parameter juice in this case consider high value YAN initial, respect Omaka Vineyard,

Table 3 Initial nitrogen status in juice GC

NH4+ (mg/L of N)	PAA (mg/L of N)	YAN (mg/L of N)
75.27	176.23	251.50

After know this value, can start to add the amino acid, so add 24 mg/L of N for single amino acid to arrive at value 200 mg/L of N.

Table 4 Quantity to add amino acid in GC, plus table BLEND1(*) and BLEND2(**)

GC Treatment	mg/750ml
CTR	-
Asp	171.1
Ser	135.1
Gln	94.0
Arg	56.0
Ala	114.5
Glu	189.2
GSH	131.7
DAP	84.9
BLEND1	***
BLEND2	****

BLEND 1***	mg/750ml
L-Aspartic Acid	12.0
Serine	9.5
L-Glutamine	15.0
L-Arginine	17.9
Alanine	24.1

BLEND 2****	mg/750ml
L-Aspartic Acid	9.6
Serine	7.6
L-Glutamine	12.0
L-Arginine	14.3
Alanine	19.2
L-Glutamic Acid	25.7
Glutathione	26.3

5.5 Ethanol:

Alcohol was determined using an Anton Parr Wine Alcolyzer. All measurements were taken in duplicate with <0.02 v/v% variation.

5.6 Glucose and Fructose:

Glucose and fructose were quantified by enzymatic assay based on the reduction of NADP. All enzymes and cofactors were purchased from Megazyme. Samples were appropriately diluted and quantified in duplicate against an 8-point standard curve (R2>0.98).

5.7 Free and Total sulphur dioxide

Free and total sulphur dioxide were measured using a chemical spectrophotometric assay purchased from Megazyme. Total sulphur was quantified by reaction with 5,5'-dithiobis-(2-nitrobenzoic acid) (Ellman's reagent) and measurement of the absorbance of the resulting chromophore at 405nm. Ellman's reagent will also react with any thiol group in the sample, so the values obtained by this methodology reflect an estimation of total sulphur dioxide, cysteine, glutathione and other sulfhydryl-containing compounds. Free sulphur dioxide was measured with a colorimetric assay involving bleaching of p-rosaniline (magenta dye) with sulphur dioxide and subsequent dye release via formaldehyde addition monitored at 575nm. All samples were analysed in duplicate and quantified against a 5-point standard curve (R^2>0.98) made from an aqueous sodium sulphite and citric acid solution.

5.8 Yeast available nitrogen (YAN):

Ammonium was quantified by enzymatic assay monitoring the deprotonation of NADPH at 340 nm. All enzymes were purchased from Megazyme; ketoglutaric acid was purchased from Sigma Aldrich. Samples were appropriately diluted (usually two-fold) and quantified in duplicate against and 5-point standard curve (R2>0.98). Primary amino acids were quantified in isoleucine (N) equivalents by the NOPA method adapted for the plate reader.

5.10 Spectrophotometer:

All spectrophotometric assays were run on a Molecular Devices Spectramax 384 Plus with a 1 cm path length cuvette reference correction. Optical density was measured directly in a UV transparent 96 well microplate at 280. 320. 420. 520 and 620 nm. Absorbance at 280 nm was used to quantify polyphenols against a gallic acid standard curve (5 point, R2 > 0.98). All measurements were recorded in duplicate.

5.11 Organic acids:

Tartaric, malic, ascorbic, citric and succinic acids were quantified on a Shimadzu Prominence HPLC system using isocratic elution with a phosphate buffer (25mM, pH 2.5) on an Allure Organic Acids Restek column (5 µm, 240 x 4.6mm). All samples were diluted five fold in a solution containing oxalic acid as an internal standard and filtered through a 0.45 µm syringe filter prior to injection. All samples were run in duplicate and quantified on a five point standard curve (R_2>0.98).

5.12 Brix, titrable acidity and pH:

Titrable acidity and pH were determined on a Mettler Toledo T70 autotitrator using an equivalence point titration. Aqueous sodium hydroxide (0.1M) was used as titrant. °Brix was determined on a Mettler Toledo RM40 refractometer. Wine samples were degassed prior to analysis.

5.13 Amino Acid Method:

Amino acids profiles were quantified on an Agilent 1200 Series HPLC using a gradient elution program of phosphate/borate buffer (10mM each, pH 8.2) and organic solvent (MeOH:MeCN:H2O, 45:45:10) on a Phenomenix Kinetix C18 column (5um, 240x4.6mm). Primary amino acids were derivatised online with o-phthaldialdehyde and 3-mercaptopropionic acid and detected by fluorescence (340nm excitation, 450nm emission). Samples were treated with iodoacetic acid to aid in the reduction of cystine. Secondary amino acids derivatised online with 9-fluorenylmethyl chloroformate and detected by fluorescence (260nm excitation, 315nm emission). A standard mix of 17 amino acids was purchased from Agilent. All standards and samples contained internal standards sarcosine (100ppm) and α-aminobutyric acid (100ppm). All samples were diluted fourfold in water and filtered through a 0.45um syringe filter prior to injection. All samples were run in duplicate and quantified on a four-point standard curve (R^2>0.98). (Henderson, J.W, et al., 2010)

5.14 Thiol analysis method:

The varietal thiols are compounds of great interest for the aroma of Sauvignon Blanc wines, derived largely from odourless precursors in the grape, they are responsible for grapefruit, passion fruit and tropical aromas as well as for green characters (Tominaga et al. 1998, 2000, Lund et al. 2009).

Analysis of the volatile thiols 3-MH, 3-MHA, and 4-MMP, was undertaken by University of Auckland, New Zealand. The method used for quantification of varietal thiols in wine: take 50

mL of wine add 500 µL of 2mM Butylated hydroxy anisole, and add 200 µL of d10-4-methyl-4-mercaptopentan-2-one and 25 µL of the internal standard mix.

After that add 500 µL of 250 mM Ethyl propiolate, stir for 2 min at 500 rpm. Adjust pH to 10.00 ± 0.05, centrifuge the sample in 50 mL falcon tubes for 10 min. When the sample preparation is finish and you switch SPE (solid-phase extraction), is important to extraction the analyte; wash sup clean cartridge with 10 mL 12% of EtOH, percolate 50 mL of wine, rinse cartridge with 5 mL MQdH2O and eluate analytes with 10 mL of dichloromethane into a 10 mL tube, dry the recovered organic phase with anhydrous Na2SO4, filter organic phase and to finish concentrate eluate under a stream of nitrogen.

The 4MMP, 3MH and 3MHA concentrations were analysed in duplicates according to the method described by (Herbst-Johnstone, M., et al 2011) with some modification. 5 mL of p-HMB (1 mM in a 0.1 M TRIS solution) was added to 50 mL of wine, followed by 0.5 mL of a 2 mM BHA solution, 50 L of 1 nmol of 4M2M2MB, 0.3 nmol of d2-3MHA and 1.5 nmol d2-3MH, used as internal standards for 4MMP, 3MHA and 3MH, respectively. Sample pH was adjusted to 7.0 ± 0.1 using 10 N, 1N NaOH and 1 N HCl as required; the sample was then loaded on a strongly basic anion exchange column (DOWEX®), previously activated using 0.1 M HCl, and ultrapure water. After elution of the sample, the column was rinsed with 50 mL of a 0.1 M sodium

acetate buffer (pH 6.0). The free thiols were collected from the thiol-HMB-complex fixed on the column by percolating 50 mL of a 50 mM L-cysteine solution (400 mg in 0.1 M sodium acetate buffer), pH 6.0. The eluate was extracted twice with dichloromethane (4 and 2 mL) after addition of 0.5 mL ethyl acetate. The organic phase was dried with Na2SO4, filtered through silanised glass wool (Supelco, Bellefonte, PA, USA), then concentrated under nitrogen flow to about 20 L.

The gas chromatographic analysis of varietal thiols is carried out using an Agilent 6890N gas chromatograph, equipped with a 7683B automatic liquid sampler, a G2614A autosampler, and a 5973 mass selective detector. Samples are placed into a tray cooled to 9°C for automated injection. The inlet temperature is held at 250°C. 2 µL of the sample is injected in pulsed split less mode, and delivered onto an Agilent HP-INNOWax capillary column using helium as carrier gas at a flow rate of 1.2 mL/min. The initial oven temperature (150°C for 2 minutes) is ramped to 250°C at rate of 10°C/min and held for 20min. The temperature of the interface line is set to 250°C. The ion source, operation in electron impact mode at 70eV, is held at 230°C. (Herbst-Johnstone, M., et, 2013)

Table 5: (Herbst-Johnstone, M., et al.,2013.)

thiol derivatives	**retention time*** [min]	**ions** [*m/z*]
d_{10}-4MMP-ETP		**240**, 142
4-MMP-ETP		230, **132**
[1-$^2H_2$]3-MHA-ETP	18.4	231, **276**
3-MHA-ETP	18.5	229, **274**
[1-$^2H_2$]3-MH-ETP	22.2	189, **234**
3-MH-ETP	22.3	187, **232**

5.15 Ester, Terpene, Norisoprenoid, Cinnamate, Phenol, Fatty Acid, Alcohol and Aldehyde Analysis:

Quantification of varietal ester, terpene, norisoprenoid, cinnamate, phenol, fatty acid, alcohol and aldehyde analysis. Weigh 3 g of sodium chloride into a 20 mL, add 10 mL of sample into the vial and add 30 µL of a standard mix solution containing the following deuterated internal standards, add 50 µL of a standard mix solution containing DL-3-octanol, 4-decanol and 3,4-dimethylphenol. Purge vial with argon and seal it with a screw cap.

Samples are placed onto the Gerstel; multiPurpose Sampler MPS2 tray. Each sample is incubated for 10 min in the Gerstel Agitator/ Stirrer AS at 45°C while agitated at 500 rpm prior to extraction. A 2 cm, 23-Gauge, 50/30 µm, DVB/CAR/PDMS fibre for Automated Holder, Gray Notched, which is pre-baked out for 5 min at 250°C in the front injection port, is exposed in the 20 mL capped vial for 60 min at 45°C. During this exposure period, the quantity of analyte extracted by the fibre is proportional to its concentration in the sample as long as equilibrium is reached. After extraction, the fibre is transferred to the rear injection port of an Angilent 7890A GC System coupled to a mass selective detector model 5975C inert XL, where desorption of the analyte in split mode at a ratio of 3:5:1 takes place for 10 min at 250°C. Helium is used as the carrier gas at a flow rate of 1mL/min. Volatiles are separated on a tandem column composed of a HP-1ms. The oven program was as follow: the initial oven temperature of 40°C was held for 5 min, giving a total run time of 90.5min. The temperature of the interface line is set to 250°C The ion source, operating in electron impact mode at 70 eV, is held at 230°C. The quadrupole temperature is set at 150°C. The aroma compounds and internal standards were detected in SIM (besides the retention time given by standard solution). Samples are placed onto the Gerstel MultiPurpose Sampler MPS2 tray (VT32-20). Each sample is incubated for 10 min in the Gerstel Agitator/Stirrer AS at 45°C while agitated at 500 rpm prior to extraction. A 2 cm, 23-Gauge, 50/30 µm, DVB/CAR/PDMS fibre for Automated Holder, Gray, Notched (SUPELCO, Bellefonte, Pennsylvania, USA; #57299_U), which is pre-baked out for 5 min (67 mm bakeout penetration) at 250°C (pressure at 48.745 kPa, total flow

rate of 70.571 mL/min, and a septum purge flow of 2 mL/min) in the front injection port (FrontS/S), is exposed in the 20 ml capped vial (31 mm vial penetration) for 60 min at 45°C. During this exposure period, the quantity of analyte extracted by the fibre is proportional to its concentration in the sample as long as equilibrium is reached. After extraction, the fibre is transferred to the rear injection port (Back PTV Inlet) of an Agilent 7890A GC System coupled to a mass selective detector model 5975C inert XL (Santa Clara, CA, USA), where desorption of the analyte in split mode at a ratio of 3.5:1 (split flow 3.5 ml/min, pressure 28.097 kPa, total flow rate of 6.5 mL/min, and a septum purge flow of 2 mL/min) takes place for 10 min (600 s) at 250°C (67 mm injection penetration). Helium is used as the carrier gas (27.881 kPa) at a flow rate of 1 mL/min. Volatiles are separated on a tandem column composed of a HP-1ms (30 m, 0.320 mm ID, 0.25 µm film) and HP-INNOWax (30 m, 0.320 mm ID, 0.25 µm film) (Agilent, USA). The oven program was as follow: the initial oven temperature of 40°C was held for 5 min, then

ramped to 200°C at a rate of 2°C/min, raised to 240°C at 80°C/min and held for 5 min, giving a total run time of 90.5 min. The temperature of the interface line is set to 250°C. The ion source, operating in electron impact mode at 70 eV, is held at 230°C. The quadrupole temperature is set at 150°C. The aroma compounds and internal standards were detected in SIM (besides the retention time given by a standard solution)

IS¤	Concentrations· in· final· sample· (µg/L)¤	¤
ethyl·butyrate-4,4,4-d_3¤	494¤	¤
ethyl·hexanoate-d_{11}¤	1178¤	¤
ethyl·octanoate-d_{15}¤	1090¤	¤
3-methylbutyl·acetate-d_3¤	1194¤	¤
n-hexyl·acetate-d_3¤	659¤	¤
2-phenylethyl·acetate-d_3¤	292¤	¤
(±)-linalool-d_3¤	76¤	¤
α-terpineol-d_3¤	75¤	¤
3-methyl-1-butyl-1,1-d_2-alcohol¤	26525¤	¤
n-hexyl-2,2,3,3,4,4,5,5,6,6,6-d_{11}-alcohol¤	1189¤	¤
2-phenyl-d_5-ethanol¤	8833¤	¤
hexanal-d_{12}¤	67¤	¤
hexanoic-d_{11}·acid¤	8253¤	¤

Figure 10 (Herbst-Johnstone, M., et al., 2013.)

6. Results and Discussion

6.1 Fermentation kinetics

For Omaka Vineyard the harvest took place on 06/04/2017, as shown in table 2, "Low YAN" is considered a low initial value of yeast assimilable nitrogen.

After the addition of amino acids and yeast, the first measure of ° Brix and temperature is carried out after 12h to monitor the measurement of alcoholic fermentation.

For Omaka it started with 20.8 ° Brix (Figure 2) the fermentation for OV lasted about 27 days, none of the theses compared was able to finish the fermentation, it turns out that Ala was the lowest value (Fig. 2),is considers dry with the value less than 3 g / L..

In fact the control and glutathione had the highest residual sugars of 56.3 g/L and 57.7 g/L respectively (Table 11) and did not reach dryness.

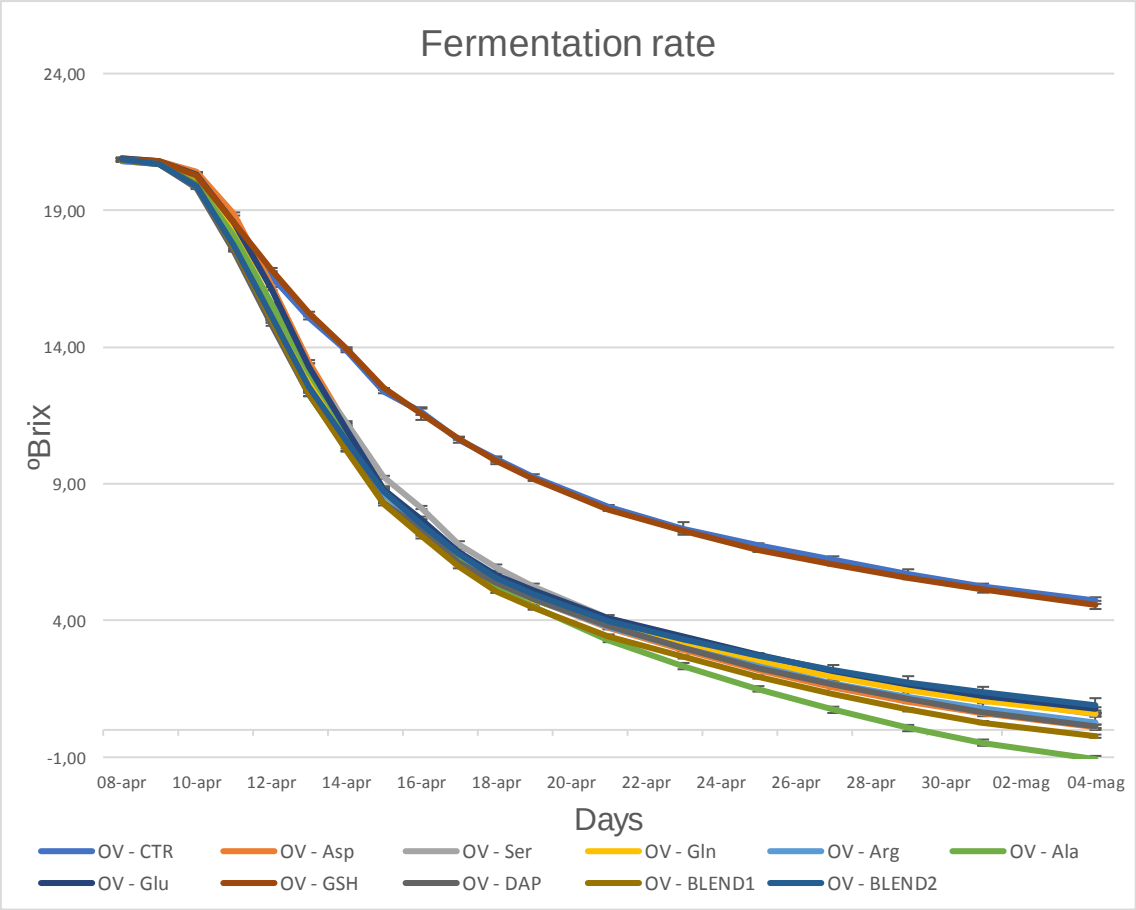


Figure 11: Monitoring Fermentation Omaka Vineyard

Gifford's Creek Vineyard grape harvest started 11/04/2017 is considered "High YAN".

At the time of harvest, can indicate it as High YAN for a high value of yeast assimilable nitrogen.

The duration of the alcoholic fermentation is about 14 days (Fig. 3) starting with 18 ° Brix.

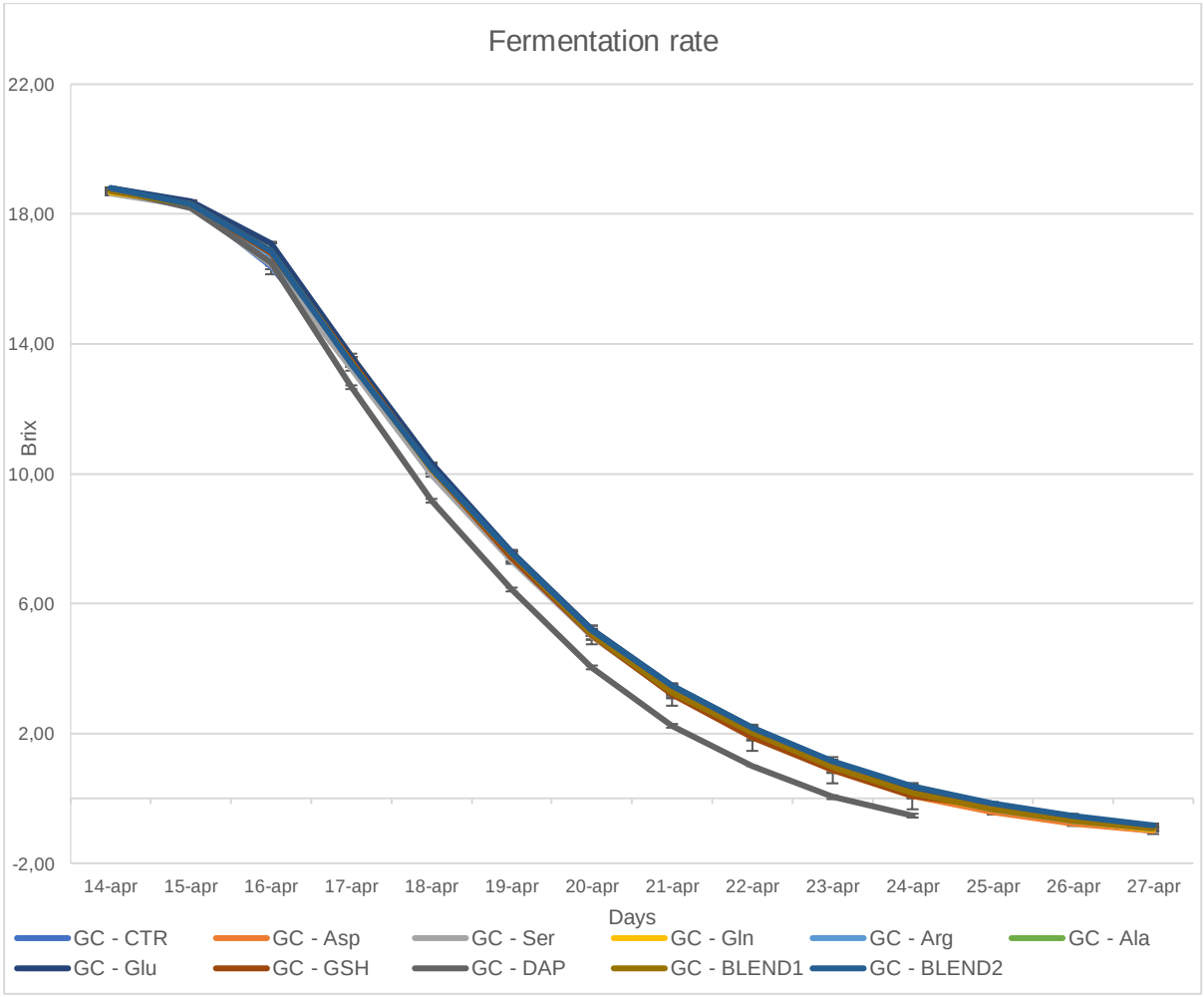


Figure 12: Monitoring Fermentation Omaka Vineyard

The two fermentation curves for GC and OV a different fermentation dynamic, because different starting PAA and YAN. From this it could understand that starting from different musts and different concentrations it significantly changes the evolution of alcoholic fermentation.

6.2Temperature Fermentation

The Temperature for Omaka Vineyard during alcoholic fermentation it is constant about between 14.80 – 15.90 ℃.

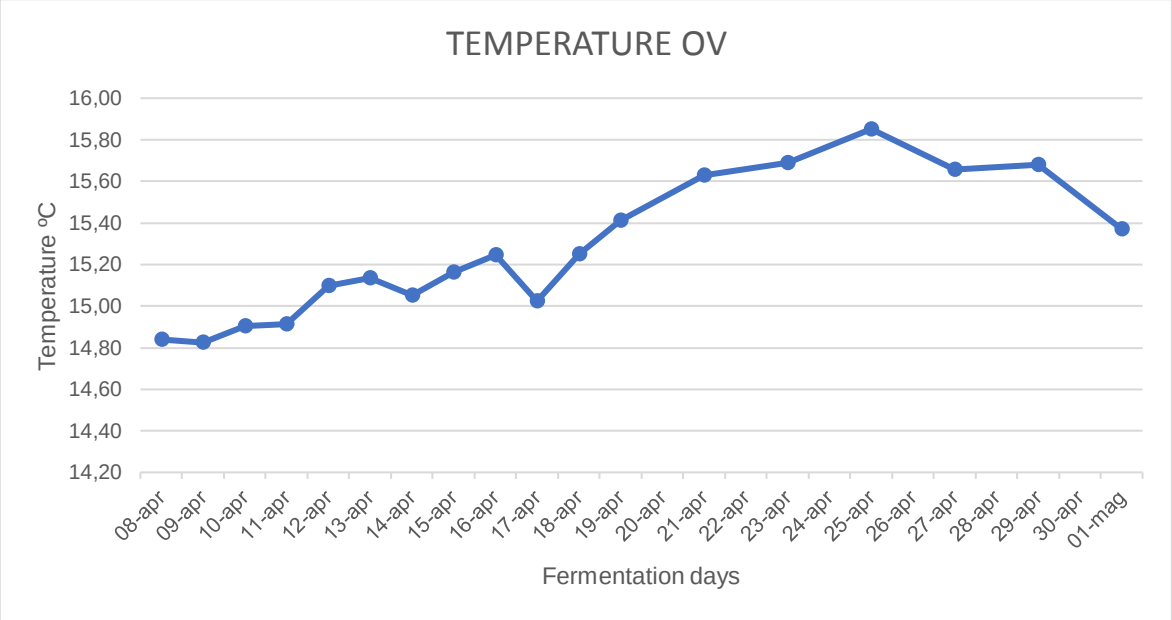


Figure 13 Temperature control during AF for OV

The Temperature for Gifford's Creek Vineyard during alcoholic fermentation is also constant about between 14.60 – 15.80 ºC.

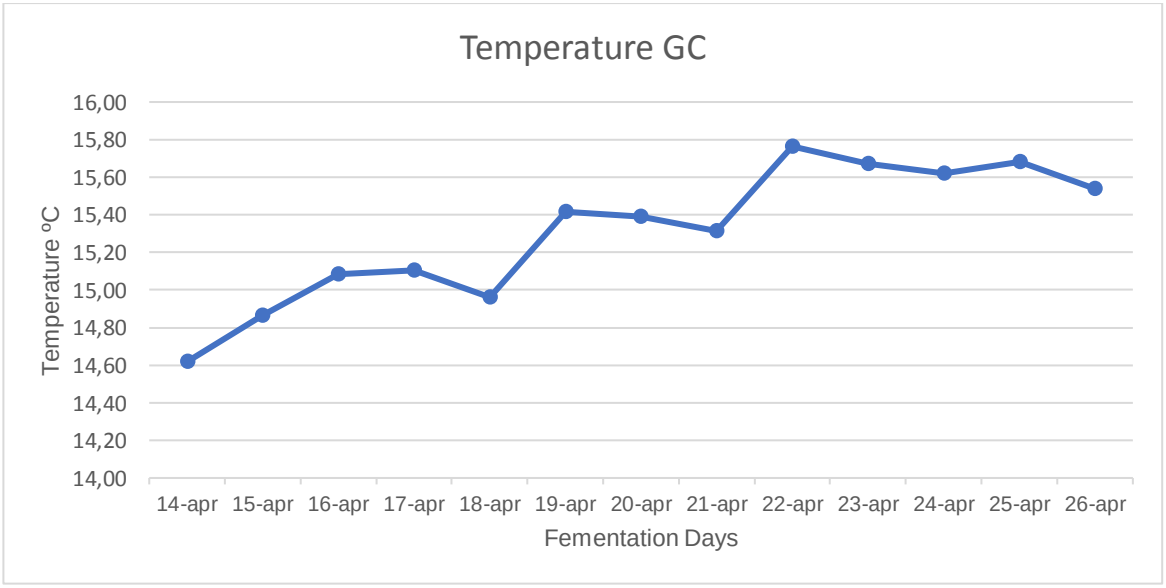


Figure 14 Temperature control during AF for GC

6.3 Value source of nitrogen

In general, grape juice presented higher concentration of amino acids and derivates than their resulting wines; (Figure13) represented the value of $NH4^{+,}$ PAA and YAN in juice OV after add single amino acid. The objective was to have the same value PAA about 200 mgN/L, but the analysis chemical read just the first nitrogen in the amino acid, understand better the real value the single amino acid is in the analysis in the profile amino acid.

Table 6: Value start source nitrogen in the juice for single treatment in OV

Vineyard	Treatment	NH4+ (mgN/L)	PAA (mgN/L)	YAN (mgN/L)
OV	CTR	15.79± 1.32 **a**	86.2± 0.57 **a**	102± 1.87 **a**
	Asp	17.34± 0.18 **b**	169.5± 2.20 **d**	186.9± 2.02 **de**
	Ser	17.35± 0.46 **b**	206.4± 6.90 **e**	223.8± 6.69 **f**
	Gln	18.04± 0.83 **bc**	149.3± 6.31 **c**	167.3± 7.11 **c**
	Arg	17.45± 0.51 **b**	120.9± 6.32 **b**	138.4± 6.21 **b**
	Ala	17.54± 0.49 **b**	210.7± 5.81 **e**	228.3± 5.38 **f**
	Glu	17.17± 0.10 **ab**	165.9± 14.27 **d**	183.1± 14.34 **de**
	GSH	17.63± 0.11 **b**	128.6± 2.86 **b**	146.2± 2.96 **b**
	DAP	64.19± 0.11 **d**	95.5± 4.36 **a**	159.7± 4.44 **c**
	BLEND1	19.14± 2.30 **c**	169.6± 0.72 **d**	188.7± 1.60 **e**
	BLEND2	17.3± 0.29 **b**	160.7± 1.95 **d**	178± 1.90 **d**
	***P**	**<.001**	**<.001**	**<.001**

And for GC after add have other value of $NH4^{+,}$ PAA and YAN in the juice, and with analysis statistic can see that there is different between the treatment.

Table 7: Value start source nitrogen in the juice for single treatment in GC

Vineyard	Treatment	NH4+ (mgN/L)	PAA (mgN/L)	YAN (mgN/L)
GC	CTR	75.27± 2.27 **ab**	191.2± 4.21 **a**	266.5± 2.06 **a**
	Asp	73.43± 4.29 **a**	199.1± 5.70 **b**	272.6± 8.26 **ab**
	Ser	80.98± 1.08 **ab**	213.1± 1.39 **c**	294.1± 2.46 **de**
	Gln	77.17± 12.04 **ab**	202.3± 7.04 **b**	279.4± 9.22 **bc**
	Arg	75.04± 3.92 **ab**	201.2± 3.14 **b**	276.3± 6.19 **ab**
	Ala	74.99± 4.48 **ab**	220.7± 3.57 **d**	295.7± 6.21 **de**
	Glu	86.37± 6.66 **b**	216.5± 5.31 **cd**	302.9± 10.07 **e**
	GSH	76.89± 3.26 **ab**	202.8± 1.88 **b**	279.7± 1.38 **bc**
	DAP	200.24± 2.18 **c**	217.5± 4.58 **cd**	417.7± 6.37 **f**
	BLEND1	83.12± 5.31 **ab**	205.4± 4.30 **b**	288.6± 1.19 **cd**
	BLEND2	86.45± 15.58 **b**	202± 2.18 **b**	288.5± 13.53 **cd**
	*P	<.001	<.001	<.001

Table of enological parameter of Omaka Vineyard

Vineyard	Treatment	Brix°	pH	TA (g/L)	Reducing Sugars	OD 280 (nm)	OD 320 (nm)	OD 420 (nm)	A420/A320 (nm)*	Polyphenols (mgGAE/L)**
OV	CTR	20.4	2.93 ab	9.713 f	212.15	6.593 a	7.39 a	0.09	0.0116 ef	208.5 a
	Asp	20.5	2.93 ab	10.03 h	214.79	6.518 a	7.294 a	0.09	0.012 f	206 a
	Ser	20.43	2.95 bc	9.55 d	213.67	7.025 b	7.755 b	0.09	0.011 def	222.7 b
	Gln	20.5	2.94 b	9.473 c	215.69	7.246 bc	7.955 bc	0.08	0.0105 a	230 bc
	Arg	20.5	2.97 cd	9.257 a	217.50	7.274 c	7.967 bc	0.09	0.0112 bcde	230.9 c
	Ala	20.5	2.98 d	9.373 b	209.91	7.226 bc	7.941 bc	0.08	0.0106 ab	229.3 bc
	Glu	20.5	2.94 b	9.837 g	219.35	7.316 c	8.027 c	0.09	0.0108 abc	232.3 c
	GSH	20.47	2.91 a	9.64 e	219.77	7.319 c	8.016 c	0.09	0.0107 abc	232.4 c
	DAP	20.5	2.99 d	9.367 b	213.22	7.297 c	7.978 bc	0.09	0.0109 abc	231.7 c
	BLEND1	20.5	2.94 ab	9.46 c	213.70	7.359 c	8.026 c	0.09	0.0110 abcd	233.7 c
	BLEND2	20.5	2.94 b	9.487 c	217.29	7.369 c	8.015 c	0.09	0.0113 cde	234 c
	*P.	0.595	<.001	<.001	0.31	<.001	<.001	0.24	<.001	<.001

Table 8: Means followed by different letters within a row are significantly different at the least significant difference level of 5 % (Fischers protected LSD)

**A420/A320 is an oxidative index; Fisher's protected LSD is not calculated as variance ratio*

***GAE = Gallic acid equivalents*

Table of enological parameter Gifford Creek Vineyard

Vineyard	Treatment	ºBrix	pH	TA (g/L)	Reducing Sugars	OD 280 (nm)	OD 320 (nm)	OD 420 (nm)	A420/A320 (nm)*	Polyphenols
GC	CTR	18.47	3.00 a	10.98 cd	188.27	5.87	6.334 bc	0.08	0.012	188.06
	Asp	18.50	2.99 a	11.01 d	186.75	5.70	6.213 a	0.08	0.013	182.56
	Ser	18.47	2.99 a	10.76 ab	191.88	5.75	6.298 ab	0.07	0.012	184.35
	Gln	18.47	2.997 a	10.85 bcd	181.88	5.78	6.318 bc	0.07	0.012	185.30
	Arg	18.47	3.007 a	10.75 ab	193.94	5.78	6.332 bc	0.07	0.012	185.20
	Ala	18.50	3.01 a	10.76 abc	189.39	5.76	6.319 bc	0.08	0.012	184.46
	Glu	18.50	3.00 a	10.87 bcd	188.09	5.78	6.349 bc	0.08	0.012	185.23
	GSH	18.50	3.00 a	10.75 ab	191.61	5.81	6.39 bc	0.07	0.012	186.33
	DAP	18.53	3.12 b	10.8 abcd	183.75	5.85	6.419 c	0.07	0.011	187.68
	BLEND1	18.50	2.99 a	10.69 ab	187.60	5.76	6.329 bc	0.07	0.012	184.64
	BLEND2	18.50	2.99 a	10.58 a	189.77	5.88	6.42 **c**	0.08	0.012	188.50
	*P	0.533	<.001	0.025	0.169	0.111	0.023	0.343	0.113	0.109

*Table 9: Means followed by different letters within a row are significantly different at the least significant difference level of 5 % (Fischers protected LSD) *A420/A320 is an oxidative index; Fisher's protected LSD is not calculated as variance ratio*

***GAE = Gallic acid equivalents*

In the juice, the chemical analysis for OV and GC vineyard set out in the Table 9 and Table 10, where don't find so much different in the juice after add the amino acid.

The addition of amino acid not change the chemical parameter in the juice, the parameter stays similar about ºBrix, pH, TA (g/L), Reducing Sugars (g/L), OD 280 (nm), OD 320 (nm), OD 420 (nm), A420/A320 (nm),Polyphenols (mgGAE/L).

Table 11 and Table 12 shows the enological parameter of the wines obtained from the different fermentation carried out. All the wines showed a similar pH between 2.99-3.1 for both vineyard; the wine obtained from the fermentation in the OV there were residual sugar so different between the single amino acid and also for wine alcohol, for Omaka Vineyard not was fast the alcoholic fermentation and not all done the fermentation "Ala" was first to finish with low value of residual sugar and after DAP and BLEND1; CTR and GSH found similar enological parameter on residual sugar (g/L), alcohol (%v/v) and pH; in Table 11 shows the Total Acidity obtained from fermentation in particularly the CTR. comparison the other treatment found differences about 1g/L.

The source of nitrogen after add in Low YAN (OV) and High YAN(GC) shows in the tables have higher differences, In fact for the OV the fermentation during about one month and for GC about 14 days, this would indicate that the addition of different quantities of amino acids to the must had a little effect on the fermentation kinetics, probably due to the fact that once the concentration of 140mgN/L of assimilable nitrogen is reached, there is variation in the rate of fermentation in response to nitrogen supplementation (Bell, et al.,2005).

The statistical analysis means that for OV there was significant differences between treatment, for the GC the results from statistical analysis explain not significant differences.

These results are interesting because in the literature don't' found more significant results about the enological parameter, because when started with Low YAN everything change during alcoholic fermentation in the wine

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Table of means and statistical relevance of wine for Omaka Vineyard

Vineyard	Treatment	Wine Alcohol(%v/v)	Reducing sugars (g/L)	Wine_pH	Wine_TA (g/L Tart)	Polyphenols (mgGAE/L)	OD 280 (nm)	A420/A320 (nm)*
OV	CTR	9.17 a	56.26 f	3.00 ab	10.13 h	209.7 ab	6.452 ab	0.018
	Asp	11.69 d	18.33 b	3.08 d	9.56 e	217.4 bc	6.684 bc	0.016
	Ser	11.38 c	23.06 d	3.06 c	9.257 c	214.8 bc	6.606 bc	0.017
	Gln	11.38 c	23.1 d	3.06 c	9.437 d	220 c	6.762 c	0.017
	Arg	11.55 d	20.64 c	3.06 c	9.167 bc	202.4 a	6.236 a	0.018
	Ala	12.29 g	8.03 a	3.06 c	9.087 ab	208.5 ab	6.418 ab	0.017
	Glu	11.31 bc	23.98 de	3.07 cd	9.91 g	220.9 c	6.79 c	0.016
	GSH	9.26 a	57.69 f	3.01 b	9.63 e	215.1 bc	6.616 bc	0.018
	DAP	11.65 de	16.39 b	2.99 a	9.75 f	216.4bc	6.654 bc	0.016
	BLEND1	11.87 f	16.24 b	3.06 c	9.047 a	213 bc	6.553 bc	0.016
	BLEND2	11.23 b	26.08 e	3.06 c	9.163 bc	215.8 bc	6.637 bc	0.016
	*P	<.001	<.001	<.001	<.001	0.04	0.04	0.453

*Table 10 Means followed by different letters within a row are significantly different at the least significant difference level of 5 % (Fischers protected LSD) *A420/A320 is an oxidative index; Fisher's protected LSD is not calculated as variance ratio*

***GAE = Gallic acid equivalents*

Table of means and statistical relevance of wine for Gifford's Creek Lane Vineyard

Vineyard	Treatment	Wine Alcohol(%v/v)	Reducing sugars (g/L)	Wine_pH	Wine_TA (g/L Tart)	Polyphenols (mgGAE/L)**	OD 280 (nm)	A420/A320 (nm)*
GC	CTR	10.96 bc	2.744	3.02 ab	10.62 a	193.92	5.999 ab	0.017
	Asp	10.97 bc	2.743	3.04 abc	10.75 a	194.58	6.018 ab	0.018
	Ser	10.96 bc	3.288	3.04 bc	10.5 a	193	5.972 ab	0.017
	Gln	10.97 bc	2.842	3.02 ab	10.62 a	191.85	5.938 ab	0.018
	Arg	10.96 bc	3.145	3.03 abc	10.5 a	191.65	5.933 ab	0.017
	Ala	10.97 bc	2.98	3.03 abc	10.8 a	193.38	6.034 ab	0.017
	Glu	10.94 b	3.606	3.02 ab	10.77 a	192.9	5.969 ab	0.017
	GSH	10.96 bc	2.881	3.01 a	10.7 a	188.68	5.846 a	0.018
	DAP	10.83 a	3.182	3.02 ab	11.6 b	199.7	6.274c	0.019
	BLEND1	10.95 bc	3.049	3.00 a	10.64 a	194.28	6.009 ab	0.018
	BLEND2	10.99 c	2.975	3.05 c	10.88 a	194.79	6.126 bc	0.019
	*P	<.001	0.719	0.041	<.001	0.069	0.023	0.127

Table 11: Means followed by different letters within a row are significantly different at the least significant difference level of 5 % (Fischers protected LSD)

**A420/A320 is an oxidative index Fisher's protected LSD is not calculated as variance ratio for Treatment*

***GAE = Gallic acid equivale*

6.4 Aroma Compounds

Esters, higher alcohols, volatile fatty acids, terpenes, norisoprenoid are important contributors to the fermentation bouquet of wine. These compounds principally arise as primary metabolites of yeast sugar and amino acid metabolism. (Teresa Garde-Cerda, et al., 2006). In the Table 13, 14, 15, 16 it can be seen all aroma compounds from Omaka Vineyard and Gifford's Creek Vineyard.

About the Omaka Vineyard it can be seen that a significant correlation ($p<0.01$) existed between the formation of single aroma compounds and the quantity of amino acids added to the must these ferments for OV did not reach dryness and therefore may influence the concentration of aroma compounds in the finished wine. Ethyl isobutyrate with aroma note strawberry, kiwi, fruity can be seen concentration for Ala treatment also for Ethyl 2-methyl butanoate with aroma note fruity and kiwi and Ethyl decanoate with expression flowery and fruity. It can be seen significant correlation in isobutyl acetate and isoamyl acetate with aroma note banana and fruity in Ala treatment production highest concentration; for β-phenylethyl acetate expression of flowery, fruity and olive possible to see per Ser treatment. For the group of Alcohols, the high concentration is with Ala treatment with isobutanol expression wine, solvent and bitter. As far as it is concerned norisoprenoids, β-damascenone with aroma note of rose concentration was not significantly effected by amino acid additions..

As far as it is concerned Gifford's Creek Vineyard it can be seen that a significant correlation in Ethyl octanoate and Ethyl acetate with aroma note sweet, ripe banana and pear with highest concentration per BLEND1 treatment. For the group of Norisoprenoid it can be seen that BLEND1 has a trend of high concentration with aroma note of rose and floral compared to the CTR but not significantly different. About Terpenes, only nerol for BLEND2 treatment had the highest concentration for Blend 1 & 2 compared to control. In Fatty Acids, BLEND1 had significantly different of octanic acid with aroma note of fatty acid and dry and dairy, probably because YAN gave different concentrations of different amino acids influencing the composition of aroma compounds.

Was made a tasting wine, all 66 sample, 33 samples for Omaka Vineyard and 33 for Gifford's Creek Vineyard, there was a majority that preferred Alanine and Serine treatment this for Omaka Vineyard, for Gifford's Creek Vineyard there wasn't a majority because the wines were similar to olfactory sensory.

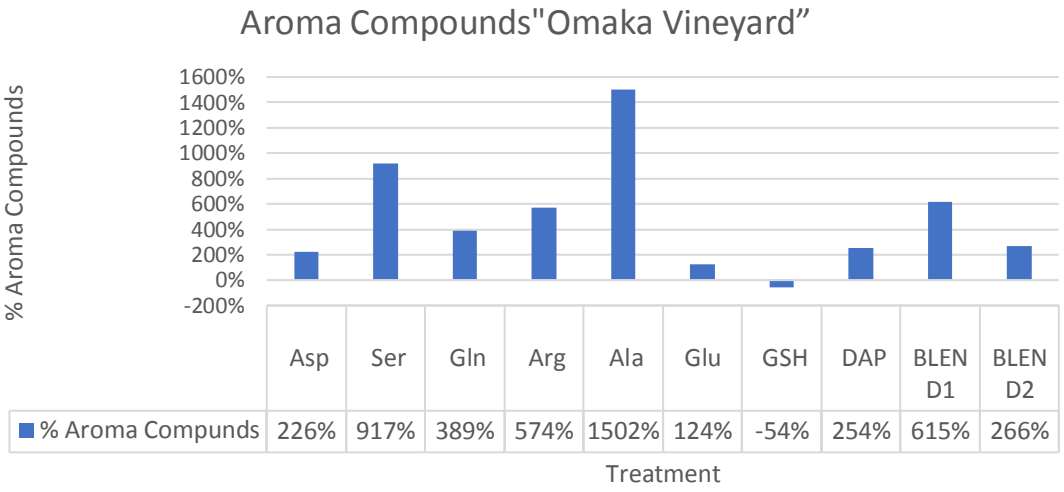

Figure 15 Comparing the aromatic compounds with the control expressed in percentage for OV

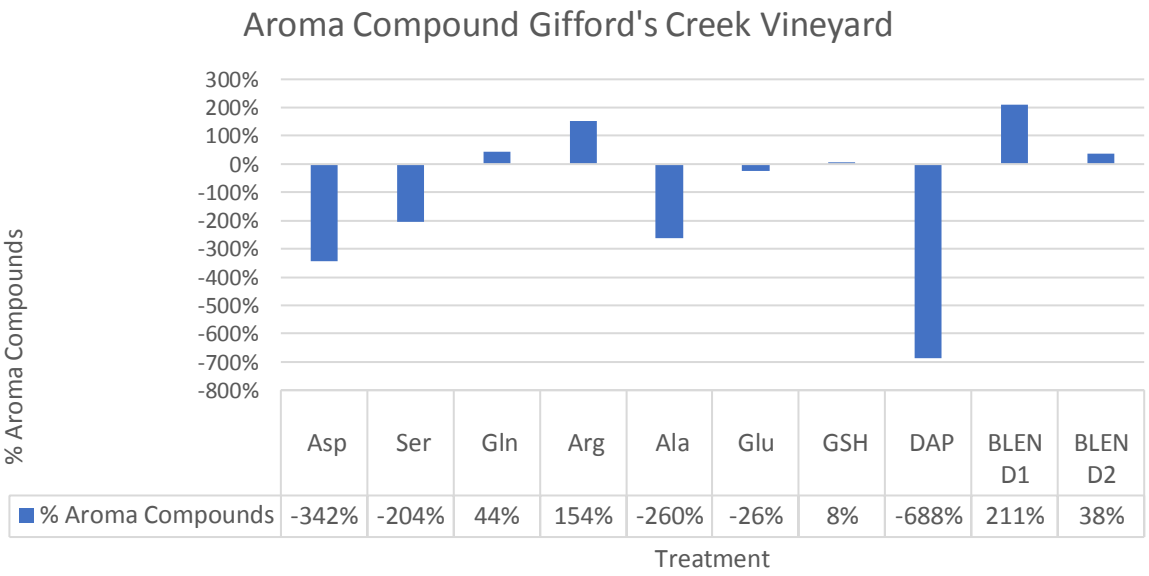

Figure 16 Comparing the aromatic compounds with the control expressed in percentage for GC

In Figures 15 and 16, the sum of all the aromatic compounds was made and then compared with the expressed control in percentage to see which amino acid has a higher production of the respective aromatic compounds. This figure shows that Alanine with 1502% and Serine 917% (Fig 15); for Gifford's Creek Blend1 with 211% but DAP -688% (Fig 16) because the juice YAN for this treatment was 410mg / L (200mg / L NH4) while the other treatments had a mean of 290mg / L YAN (77mg / L NH4) and this probably explains the faster fermentation duration for DAP.

Table 12 Aroma Compounds Omaka Vineyard for each treatment I

Vineyard: OV Low YAN	CTR	Asp	Ser	Gln	Arg	Ala	Glu	GSH	DAP	BLEND1	BLEND2	*P
Ethyls esters (µg/L)												
ethyl isobutyrate	8.36 a	9.66 a	14.73 a	8.4 a	10.64 a	22.22 b	7.5 a	9.1 a	10.31 a	10.12 a	9.18 a	0,025
ethyl butanoate	292	469	470	472	500	489	460	302	417	501	421	0,05
ethyl 2-methyl butanoate	0,792	0,991	0,877	0,521	0,842	1,355	0,461	0,872	0,96	0,698	0,665	0,234
ethyl isovalerate	1,62	1,65	2,23	1,22	1,48	1,8	1,22	1,78	1,99	1,3	1,2	0,574
ethyl hexanoate	1332	1325	1507	1530	1610	1533	1393	1347	1309	1606	1388	0,735
ethyl octanoate	798	981	1077	1221	1320	1199	1107	855	1152	1391	1186	0,241
ethyl decanoate	172	210	241	288	336	310	278	167	273	340	266	0,094
Acetate Esters (µg/L)												
ethyl acetate	27021 a	36873 abc	47101 abcd	47135 abcd	52489 bcd	62051 d	44017 abcd	30178 ab	26408 a	58015 cd	39805 abcd	0,044
isobutyl acetate	42 a	70.9 abc	127.3 d	72.3 abc	81 abc	253.4 e	72.1 abc	45.1 ab	51.9 ab	103.6 cd	88.9 bcd	<.001
isoamyl acetate	3499 a	5680 bc	7967 d	5826 c	5630 bc	6379 cd	5580 bc	3857 ab	4716 abc	6039 c	5127 abc	0,008
hexylacetate	229,4	286,8	296,7	302,7	314,5	282	290,6	230	250,4	303,7	282,9	0,43
cis-3-hexenyl acetate	14,59	20,32	17,42	18,92	19,59	18,87	19,17	14,51	17,33	20,32	18,22	0,167
β-phenylethyl acetate	324.3 de	196.7 ab	411.5 f	211.5 abc	254.5 bcd	386.9 ef	151.5 a	281.4 cd	177.6 ab	225.1 abc	216.5 abc	<.001
Alcohols (µg/L)												
isobutanol	13490 a	12122 a	25921 b	12697 a	14386 a	39811 c	11776 a	11660 a	10525 a	16624 a	15202 a	<.001
1-butanol	1024 ab	1425 bcd	1049 abc	1477 cd	1419 bcd	1961 e	1464 bcd	914 bcd	1368 bcd	1653 de	1456 bcd	0,005
isoamylalcohol	134111 cd	90555 ab	153167 d	96845 ab	99606 ab	113262 bc	86535 ab	115694 bc	73370 a	95315 ab	90702 ab	0,002
phenylethyl alcohol	20516 f	4186 a	12651 d	6272 ab	7921 bc	9688 cd	4674 a	16043 e	5185 e	6509 ab	6814 abc	<.001

Table 13 Aroma Compounds Omaka Vineyard for each treatment II

Vineyard: OV Low YAN	CTR	Asp	Ser	Gln	Arg	Ala	Glu	GSH	DAP	BLEND1	BLEND2	*P
Norisoprenoids (µg/L)												
β-damascenone	6,84	5,68	5,95	6,38	6,09	4,61	4,8	6	7,38	5,47	5,83	0,762
β-ionone	0,7268	0,7195	0,7179	0,7292	0,7177	0,7112	0,7045	0,7288	0,7436	0,716	0,7162	0,896
Terpenes (µg/L)												
cis/trans-rose-oxide	0,5099	0,5349	0,5281	0,521	0,5223	0,5369	0,5189	0,5099	0,5591	0,5198	0,5212	0,738
α-terpineol	7,47	6,51	6,84	6,83	6,62	6,58	6,35	7,62	7,39	6,29	6,79	0,998
β-citronellol	4.31 abc	5.54 cde	5.87 de	4.3 abc	4.79 abcd	6.36 e	4.94 bcde	3.37 a	5.6 cde	5.15 bcde	3.78 ab	0,014
nerol (cis-geraniol)	26.8 a	37.4 abc	51.3 cd	46.3 cd	41.8 abcd	44.7 cd	38.9 abc	27 ab	56.5 d	43.8 bcd	38.8 abc	0,035
C6 Alcohol (µg/L)												
hexanol	619	423	569	463	497	480	414	501	372	450	430	0,168
Fatty acids (mg/L)												
hexanoic acid	5,95	4,6	7,05	6,55	6,88	6,13	5,62	4,54	5,06	6,22	5,77	0,441
octanoic acid	7,3	8,05	7,83	9,17	8,88	9,1	8,11	8,44	9,52	7,09	8,67	0,443
decanoic acid	7,83	6,55	6,31	7,11	6,66	6,27	7,1	8,12	7,44	5,59	7,07	0,118
Cinnamates (µg/L)												
ethyl (di)hydrocinnamate	0.601 e	0.5212 abc	0.5385 cd	0.5087 a	0.5321 bc	0.5523 d	0.5058 a	0.591 e	0.5187 abc	0.5055 a	0.516 ab	<.001
Thiols (ng/L)												
3MHA	844	712	814	1016	728	819	634	724	804	991	776	0,866
3MH	2495	2903	2482	2574	2925	2223	2153	3070	2499	2601	2697	0,493

Table 14 Aroma Compounds Gifford Creek Vineyard for each add amino acid I

Vineyard GC High YAN	CTR	Asp	Ser	Gln	Arg	Ala	Glu	GSH	DAP	BLEND1	BLEND2	*P
Ethyls esters (µg/L)												
ethyl isobutyrate	9,18	7,42	9,18	10,11	12,83	8,24	9,56	11,46	4,57	10,91	11,04	0,313
ethyl butanoate	727	607	649	702	734	566	625	712	510	684	605	0,612
ethyl 2-methyl butanoate	0,691	0,627	0,67	0,723	1,137	0,652	0,753	0,937	0,357	0,825	0,958	0,237
ethyl isovalerate	1,42	1,21	1,46	1,61	2,23	1,31	1,66	1,95	0,8	1,74	1,98	0,311
ethyl hexanoate	2092	1712	1765	1954	1958	1683	1812	1937	1682	2041	1783	0,79
ethyl octanoate	1031 abc	929 a	894 a	1087 abcd	1157 abcd	979 ab	1106 abcd	1315 cd	1009 ab	1356 d	1252 bcd	0,041
ethyl decanoate	203,6	195,4	174,9	235,3	231	219,7	242,4	260,9	211,7	318,6	270,1	0,054
Acetate Esters (µg/L)												
ethyl acetate	90673	73782	62102	103550	84615	55353	77654	84561	55656	112625	68116	0,401
isobutyl acetate	192,5	153,4	172	184,7	190,6	160,1	167,1	180,5	92,5	194	155,4	0,086
isoamyl acetate	11177	9591	9920	11336	10699	8804	10038	10715	6923	11507	9344	0,378
Hexylacetate	569	452	503	532	564	466	518	554	452	557	511	0,691
cis-3-hexenyl acetate	36,1	30,7	32,9	39,9	36,7	31,4	34,5	34,9	32,3	41,5	34,3	0,816
β-phenylethyl acetate	316.5 c	271.4 b	257.4 ab	275.5 bc	286.8 bc	297.1 bc	287.6 bc	275.4 bc	224.6 a	279.4 bc	292.4 bc	0,042
Alcohols (µg/L)												
Isobutanol	22530 b	19475 b	21155 b	19175 b	20905 b	21279 b	20998 b	20247 b	9836 a	20782 b	19717 b	0,001
1-butanol	2515	2327	2232	2252	2325	2363	2394	2374	2345	2398	2335	0,983
Isoamylalcohol	135574 b	122346 b	117648 b	122243 b	123932 b	118165 b	130854 b	122519 b	81567 a	122840 b	122749 b	0,002
phenylethyl alcohol	7766 c	6769 bc	6459 b	6873 bc	6863 bc	7201 bc	7263 bc	6685 bc	4143 a	6811 bc	7117 bc	<.001

Table 15 Aroma Compounds Gifford Creek Vineyard for each add amino acid II

Vineyard GC High YAN	CTR	Asp	Ser	Gln	Arg	Ala	Glu	GSH	DAP	BLEND1	BLEND2	*P
Norisoprenoids (µg/L)												
β-damascenone	5,6	4,88	6,24	7,22	6,79	5,75	7,26	6,07	6,5	7,15	7,42	0,22
β-ionone	0,768	0,7426	0,7742	0,7981	0,7837	0,7631	0,7926	0,7726	0,7577	0,7971	0,7938	0,104
Terpenes (µg/L)												
cis/trans-rose-oxide	0,5045	0,4892	0,5039	0,4968	0,5171	0,5001	0,5238	0,509	0,4907	0,499	0,5312	0,099
α-terpineol	12,84	11,55	12,63	13,95	14,2	12,86	15,02	13,33	12,6	13,72	16,75	0,099
β-citronellol	4,057	3,445	4,354	4,434	4,009	3,732	4,356	3,948	2,85	4,417	4,069	0,059
nerol (cis-geraniol)	38.44 abc	32.83 a	39.64 abcd	42.48 bcd	41.55 bcd	37.66 abc	45.78 cd	38.71 abc	34.55 ab	44.37 cd	48.15 d	0,034
C6 Alcohol (µg/L)												
hexanol	535	438,3	444,3	447,5	486,7	461,8	489	465,8	351,1	441,4	483,2	0,053
Fatty acids (mg/L)												
hexanoic acid	5,8	4,78	4,69	4,84	5,08	5,09	5,26	4,76	4,22	4,92	5,31	0,406
octanoic acid	10.62 bcd	10.31 bcd	10.72 bcd	11.82 cd	10.91 bcd	10 abc	9.58 abc	10.07 bc	7.38 a	12.78 d	8.56 ab	0,027
decanoic acid	6,97	6,81	7,54	8,39	7,55	7,35	6,68	7,21	6,39	9,16	6,86	0,099
Cinnamates (µg/L)												
ethyl (di)hydrocinnamate	0.5503 bc	0.5479 bc	0.5375 b	0.5374 b	0.548 bc	0.5395 bc	0.5509 bc	0.5405 bc	0.516 a	0.5416 bc	0.5527 c	0,001
Thiols (ng/L)												
3MHA	1234	1186	1220	1140	1120	1058	1042	909	956	1211	993	0,82
3MH	2637	2640	2632	2658	2751	2620	2494	2325	2620	2591	2610	0,213

Table 16 Organuc Acids for Omaka Vineyard all express in g/L

Vineyard OV	Gluconic (g/L)	Tartaric (g/L)	Malic (g/L)	Citric (g/L)	Ascorbic (g/L)
CTR	3,728 f	7,033	3,949 abc	0,505 de	ND
Asp	3,386 d	6,985	3,863 ab	0,519 e	0,000
Ser	3,577 e	6,940	3,794 a	0,503 cde	0,001
Gln	3,225 bc	7,057	3,844 ab	0,498 cd	0,001
Arg	3,075 a	6,942	3,832 ab	0,470 a	0,002
Ala	3,142 ab	7,095	3,991 bcd	0,483 abc	0,016
Glu	4,538 g	6,952	4,048 cde	0,475 ab	0,001
GSH	3,675 ef	6,911	4,155 de	0,466 a	0,003
DAP	3,710 f	7,021	4,184 e	0,474 ab	0,001
BLEND1	3,322 cd	6,835	4,208 e	0,470 a	0,001
BLEND2	3,238 bc	7,093	3,843 ab	0,493 bcd	0,002
P*	0.000	0.7723	0.0000	0.0002	0.6679

Table 17 Organic Acids analysis for Gifford's Creek Vineyard all express in g/L

Vineyard GC	Gluconic(g/L)	Tartaric(g/L)	Malic (g/L)	Citric (g/L)	Ascorbic(g/L)
CTR	3,053 ab	7,057	5,751	0,655 abc	ND
Asp	3,012 ea	6,943	5,633	0,645 a	ND
Ser	3,046 ab	6,935	5,697	0,647 a	ND
Gln	3,137 ab	6,398	5,722	0,651 ab	0,057 a
Arg	3,166 ab	6,737	5,772	0,659 bcd	0,058 b
Ala	3,109 ab	7,016	5,694	0,689 d	0,057 b
Glu	3,416 ec	6,901	5,815	0,689 d	0,058 b
GSH	3,056 ab	6,672	5,626	0,650 ab	0,057 b
DAP	3,097 ab	6,621	5,712	0,679 cd	0,057 b
BLEND1	3,160 ab	6,911	5,722	0,677 cd	0,057 b
BLEND2	3,175 eb	6,878	5,749	0,685 d	0,058 b
P*	0.0034	0.8330	0.9731	0.0023	0.000

Statistical differences were seen, during the analysis of the organic acids of the wine by HPLC (Table 17) in particular for Gluconic, Malic and Citric. Glu Treatment there is the highest quantity of Gluconic in comparison with other treatment

Gifford's Creek Vineyard not statistical differences were seen not like Omaka Vineyard, the concentration express in gram of litre of organic acid for Tartaric acid between 6.6 -7 (g/L) and for Malic between 5 – 5.5 (g/L).

6.5 Amino Acids Profile

6.5.1 Juice

Analyse the results from Juice OV and GC about Amino Acids profile, Table 19 and 20.

Their additions do not change the quantitative values of other amino acids by looking at the control there is no real alteration. In juice additions increase the desired amounts of the respective amino acid.

Now let's see if there has been their increment of the individual amino acids, let's start with GC how much the increases are by subtraction the control:

- Aspartic Acid, the concentration is 809 comparison the CTR is 818, if want the difference is -9 µmol/L, the concentration is high compared to the other treatments but no increase compared to CTR, the possible explanation is that after addition the solubilization was not complete;
- Glutamic Acid is possible to see the concentration is 2196 µmol/L and the CTR is 749 µmol/L so there is a increase of concentration about 1447 µmol/L ;
- Serine the value found in the juice is 2392 µmol/L and the CTR 701 µmol/L there is an increase 1691 µmol/L;
- Glutamine is 1898 µmol/L and the CTR is 1153 µmol/L comparison the CTR is 745 µmol/L more highest;
- Arginine has been seen 4640 µmol/L and the CTR 3870 µmol/L with a greater concentration than 770 µmol/L;
- Alanine has 3850 µmol/L and CTR 1920 µmol/L there is an increase from this amino acid of 1930 µmol/L
- Glutathione is 710 µmol/L the concentration can see an increase.

For OV the addition of single amino acids is higher when compared to GC. Results for OV, how much the increases are by subtraction the control:

- Aspartic Acid has been seen 6930 µmol/L and the CTR concentration is 389 µmol/L there is a increase about 6541 µmol/L ;
- Glutamic Acid is 6670 µmol/L and the value of CTR is 558 µmol/L an increase of 6112 µmol/L;
- Serine is 8649 µmol/L and CTR 284 µmol/L were seen an increase from Serine of 8365 µmol/L;
- Glutamine is 4494 µmol/L – 399 µmol/L = 4095 µmol/L;
- Arginine has been 2916 µmol/L and the CTR is 788 µmol/L is possible to see an increase from Arginine
- Alanine has 9619 µmol/L comparison the CTR with 784 µmol/L, so Ala has an increase 8835 µmol/L;

- Glutathione is 3272 µmol/L and the CTR has a value 141 µmol/L with a greater concentration than 3131 µmol/L.

But there are some exceptions when adding GSH there is a net change in Histadine that compared to the control there is a difference of 500 µmol/L; (Table 19) also an increase in Cystine, and Isoleucine was observed. The increase in histadine can be explained when GSH is added four derivatives are produced, some of which co-elute with other amino acids so the GSH spike just produces an artifact that coelutes with histadine rather than actually increasing it.

In the case of DAP addition there is an increase in and Isoleucine 500 µmol/L compared to CTR.

Instead, for the addition of Glutamic Acid there is an increase in Tyrosine concentration of 20 µmol/L higher than the concentration of CTR.

In the case of Gifford's Creek Vineyard (Table 20) starting from a high YAN level, with low added quantities, for GC the added quantities increase the concentration of the single amino acid. With some exceptions, with the addition of Glutathione there is an increase of Histidine 500 µmol/L higher than CTR (increase in histidine is explained above), including Cystine, and Isoleucine as seen for Omaka Vineyard, this increase in these respective amino acids is confirmed.

With the addition of DAP there is an increase in Cystine.

For Alanine, can be seen there is an increase in Valine.

6.5.2 Wine

As we see in the (Tables 21 & 22), in the wine the amino acid profile is really interesting by looking at the individual treatments,

Let's start for GC, how much amino acid was used during fermentation by subtracting wine concentration from juice concentration for each amino acid:

- Aspartic Acid the yeast during alcoholic fermentation took nearly all concentration because the quantity is <25 µmol/L; The yeast utilized 784 µmol/L during alcoholic fermentation.
- Glutamic Acid from juice with 2196 µmol/L in the wine were seen 438.9 µmol/L, so the yeast took about 1757 µmol/L during AF;
- Serine the concentration from juice is 2392 µmol/L and in the wine is119.4 µmol/L, the yeast took during AF about 2273 µmol/L;
- Glutamine in the juice the concentration is 1898 µmol/L and in the wine 516.2 µmol/L, in the end took about 1382 µmol/L;
- Arginine the concentration in the juice is 4640 µmol/L and in the wine 2325 µmol/L, the yeast took about 2315 µmol/L;

- Alanine is 3850 µmol/L and in the wine 395.2 µmol/L, the yeast tok almost all cocentration 3455 µmol/L;
- Glutathione is 710 µmol/L from juice to wine is 576, the yeast took small quantity134 µmol/L.

In Arginine were seen the highest concentration of aroma compound also in BLEND1.

For OV, how much amino acid was used during fermentation by subtracting wine concentration from juice concentration for each amino acid:

- Aspartic Acid the yeast took almost all concentration during alcoholic fermentation from 6930 µmol/L in the juice to 28.49 µmol/L in the wine;
- Glutamic Acid the yeast tool almost all concentration during AF from 6670 µmol/L in the juice to101.9 µmol/L in the wine;
- Serine is 8649 µmol/L in the juice and in the wine were seen 1988 µmol/L;
- Glutamine is 4494 µmol/L in the juice and in the wine is 186.4 µmol/L
- Arginine is 2916 µmol/L in the juice and in the wine has been analyzed 96.58 µmol/L;
- Alanine is 9619 µmol/L in the juice and in the wine the value is 953.1, the yeast took 8665 µmol/L,;
- Glutathione is 3272 µmol/L in the juice and in the wine is 2505 µmol/L.

In Alanine treatment were seen the highest concentration in Aroma compounds.

while talking about Omaka Vineyard in the respective additions there are residues but at different concentrations, the one with a higher concentration is Glutathione with 2505 µmol/L, not preferred by yeast, a less residual is Aspartic Acid with 28 µmol/L and then Arginine with 96 µmol/L and Glutamic Acid with 101 µmol/L. For Gifford's Creek that with high concentration is Arginine with 2325 µmol/L however in the juice it had a high concentration around 4640 µmol/L but with the lowest concentration is Aspartic Acid <25.

DAP treated juice consumed substantially less amino acids than both the CTR and other treatment; some amino acids additions resulted in the yeast consuming much lager quantities of that amino acids, Serine and Alanine were good examples of this. Others didn’t : Arginine spike only resulted in modest increase in the amount of arginine consumed.

The control treatment for OV which had a low juice nutrition status (low YAN) utilised all amino acids (21 in number) during fermentation with the exception of GSH and proline (known not to be used by yeast). The control at GC with higher juice nutrition status (high YAN) utilised all amino acids (12 in number) except for glutamic acid, GSH, serine, glutamine, histadine, glycine, arginine, alanine, leucine, lysine and proline (known not to be used by yeast).

Table 18 Amino Acids profile Omaka Vineyard expressed in µmol/L – JUICE

OV Vineyard Juice/ µmol/L	CTR	Asp	Ser	Gln	Arg	Ala	Glu	GSH	DAP	BLEND1	BLEND2	P*
Aspartic Acid	389 b	**6.930 f**	339 a	327 a	334 a	345 a	429 c	424 c	430 c	901 e	798 d	<0.001
Glutamic Acid	558 bc	534 ab	517 a	537 ab	512 a	522 ab	**6.670 f**	595 c	585 c	1.744 e	1.619 d	<0.001
GSH	141 c	114 ab	114 ab	128 bc	133 bc	131 bc	99 a	**3.272**	184	143	648	<0.001
Asparagine	7,60	9,33	7,79	7,47	7,52	8,08	8,78	8,87	7,48	8,56	12,34	0,34
Serine	284 b	285 b	**8.649 f**	325 c	292 b	289 b	279 b	252 a	254 a	880 e	796 d	<0.001
Glutamine	399 b	436 b	394 b	**4.494 d**	411 b	404 b	377 b	199 a	425 b	921 c	848 c	<0.001
Histadine	104 bc	103 bc	72 ab	114 c	84 abc	74 ab	59 a	**659 e**	107 bc	74 ab	195 d	<0.001
Glycine	23,0	28,0	21,8	32,1	17,9	20,6	26,5	26,8	**50,7**	30,1	33,0	0,067
Threonine	360 cd	350 bcd	322 a	328 ab	321 a	346 abc	345 abc	336 abc	374 d	340 abc	334 ab	0,010
Arginine	788 bc	816 cde	838 ef	859 f	**2.916 i**	834 def	794 bcd	726 a	760 ab	1.107 h	991 g	<0.001
Alanine	784 a	787 a	786 a	797 a	790 a	**9.619 d**	802 a	780 a	792 a	2.775 c	2.439 b	<0.001
GABA	0,193 ab	0,208 bc	0,226 cde	0,230 e	0,228 de	0,225 cde	0,183 a	0,198 ab	0,209 bcd	0,208 bc	0,205 b	0,001
Tyrosine	19,1 a	19,2 a	19,5 a	18,2 a	18,4 a	19,6 a	**47,3 b**	17,2 a	16,5 a	15,8 a	20,2 a	0,017
Valine	136 bc	128 abc	120 a	125 ab	120 a	131 abc	137 bc	134 bc	137 bc	139 cd	151 d	0,006
Methionine	30,6	23,1	22,9	27,5	27,8	37,2	25,4	22,1	22,7	21,7	32,0	0,37
Cystine	1,4 a	7,3 a	4,4 a	4,5 a	2,7 a	**25,5 b**	4,5 a	**28,2 b**	4,9 a	5,3 a	**28,7 b**	<0.001
Tryptophan	6 a	11 a	14 a	10 a	12 a	11 a	**271 b**	**373 b**	**315 b**	**338 b**	**340 b**	<0.001
Phenylalanine	122	110	109	109	109	110	112	105	107	113	115	0,35
Isoleucine	72 a	81 a	77 a	77 a	77 a	78 a	82 a	**614 b**	**578 b**	**600 b**	**554 b**	<0.001
Leucine	94,9	90,4	90,1	90,1	90,2	89,9	91,8	89,3	89,1	92,0	93,4	0,18
Lysine	30,5 b	29,9 b	29,5 b	31,5 b	29,7 b	31,5 b	31,6 b	38,2 c	25,7 a	25,7 a	25,7 a	<0.001
Proline	1.920 cd	2.090 d	2.000 d	1.840 cd	1.820 bcd	1.860 cd	1.910 cd	1.840 cd	1.630 abc	1.470 a	1.520 ab	0,011

Table 19 Amino Acids profile Gifford Creek Vineyard expressed in µmol/L - JUICE

GC Vineyard Juice/ µmol/L	CTR	Asp	Ser	Gln	Arg	Ala	Glu	GSH	DAP	BLEND1	BLEND2	P*
Aspartic Acid	818 e	**809 e**	789 e	546 d	255 ab	245 ab	224 a	250 ab	231 a	**373 c**	334 bc	<0.001
Glutamic Acid	749 c	742 c	724 bc	729 bc	731 bc	727 bc	**2.196 e**	706 ab	686 a	**1.007 d**	1.032 d	<0.001
GSH	158 a	143 a	143 a	153 a	160 a	146 a	148 a	**710 d**	191 b	**148 a**	251 c	<0.001
Asparagine	43,3 b	51,2 bc	42,4 ab	41,9 ab	29,6 a	42,3 ab	42,2 ab	58,9 c	42,5 ab	**45,3 b**	43,4 b	0,034
Serine	701 ab	700 ab	**2.392 d**	709 ab	700 ab	681 a	697 ab	722 b	711 ab	**797 c**	778 c	<0.001
Glutamine	1.153 ab	1.088 ab	1.077 a	**1.898 e**	1.184 abc	1.197 abc	1.200 abc	1.254 bcd	1.336 cd	**1.143 ab**	1.422 d	<0.001
Histadine	22,2 e	19,0 de	17,8 cde	12,2 bc	5,0 a	5,1 a	4,5 a	13,3 bcd	9,6 ab	**4,0 a**	13,0 bcd	<0.001
Glycine	79,5 d	68,1 cd	62,4 cd	71,1 d	49,5 bc	37,6 ab	38,9 ab	58,7 cd	35,1 a	**38,3 ab**	60,9 cd	<0.001
Threonine	807 g	790 fg	776 ef	765 e	726 cd	707 ab	700 a	720 bc	706 ab	**728 cd**	742 d	<0.001
Arginine	3.870 ab	3.800 a	3.840 a	3.900 abc	**4.640 f**	4.090 de	4.010 bcd	4.030 cde	4.020 cd	**4.170 e**	4.080 de	<0.001
Alanine	1.920 a	1.890 a	1.910 a	1.950 ab	2.100 d	**3.850 g**	2.050 cd	2.050 cd	2.010 bc	**2.410 f**	2.270 e	<0.001
GABA	0,253 b	0,254 b	0,259 bcd	0,258 bc	0,267 cde	0,267 de	0,274 ef	0,277 f	0,302 g	**0,237 a**	0,237 a	<0.001
Tyrosine	37,8	37,2	36,3	35,6	35,3	34,7	34,9	38,1	38,8	**43,3**	38,1	0,091
Valine	6,72 ab	5,91 ab	6,64 ab	8,12 ab	8,65 ab	**16,99 c**	11,98 bc	7,92 ab	10,03 b	**3,77 a**	7,88 ab	0,023
Methionine	159	149	123	127	141	134	130	181	172	**110**	113	0,062
Cystine	166 a	155 a	153 a	150 a	160 a	151 a	157 a	**277 b**	**359 c**	**398 d**	**415 d**	<0.001
Tryptophan	70 ab	48 a	48 a	156 bc	**381 d**	**368 d**	**389 d**	45 a	**256 c**	66 d	55 d	<0.001
Phenylalanine	150 fg	148 efg	148 defg	146 bcde	146 cdef	144 abcd	142 ab	144 abcd	150 g	144 abc	142 a	0,001
Isoleucine	96 e	94 de	91 bcde	89 bcd	86 b	88 bcd	77 a	92 cde	118 f	97 e	88 bc	<0.001
Leucine	106 d	105 d	104 d	97 c	85 b	87 b	76 a	105 d	101 cd	99 c	99 c	<0.001
Lysine	22	22	23	24	36	26	36	31	33	22	22	0,13
Proline	1.170 a	1.130 a	1.100 a	1.160 a	1.360 c	1.350 bc	1.340 bc	1.200 ab	1.140 a	1.240 abc	1.200 ab	0,010

Table 20 Amino Acids profile Omaka Vineyard expressed in µmol/L – Wine

OV Vineyard Wine/ µmol/L	CTR	Asp	Ser	Gln	Arg	Ala	Glu	GSH	DAP	BLEND1	BLEND2	P*
Aspartic Acid	<25	**28.49 b**	64.46 c	<25	<25	<25	<25	ND	<25	<25	<25	<0.001
Glutamic Acid	<25	94.68 e	142.4 h	98.2 ef	69.43 b	113.8 g	**101.9 a**	29.99 a	79.77 c	85.96 d	64.99 b	<0.001
GSH	95.42 ab	123.9 b	102.9 ab	128.4 b	72.9 a	96.27 ab	118.7 b	**2505 d**	115.8 ab	96.36 ab	682.4 c	<0.001
Asparagine	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	
Serine	ND	ND	**1988**	<25	<25	<25	<25	<25	<25	<25	<25	<0.001
Glutamine	ND	112.6 d	366.8 f	**186.4 e**	26.99 a	175.8 e	93.65 c	<25	71.56 b	84.47 bc	<25	<0.001
Histadine	ND	ND	ND	60.15 a	42.26 a	45.91 a	63.53 a	575.5 c	55.71 a	55.14 a	113.1 b	<0.001
Glycine	ND	65.44 c	47.3 a	65.98 c	47.5 ab	86.68 d	62.06 bc	ND	65.97 c	59.09 abc	48.84 ab	<0.001
Threonine	ND	ND	85.65	<25	<25	<25	<25	<25	<25	<25	<25	
Arginine	<25	80.8 de	59.2 a	84.86 de	**96.58 f**	77.98 cd	87.74 ef	69.79 bc	107.6 g	95.1 f	65.08 ab	<0.001
Alanine	<25	236.6 e	492.4 f	245.9 e	177.8 c	**953.1 g**	249.1 e	29.78 a	210.2 d	210.9 d	147.8 b	<0.001
GABA	ND	<25	<25	<25	<25	<25	<25	ND	<25	<25	ND	
Tyrosine	<25	<25	35.14	<25	<25	<25	28.39	ND	27	28.86	<25	0.1037
Valine	<25	<25	65.72	27.36	<25	41.07	<25	ND	<25	<25	ND	0.1530
Methionine	ND	ND	ND	<25	<25	<25	<25	ND	<25	<25	ND	
Cystine	ND	ND	ND	<25	<25	<25	<25	ND	<25	<25	ND	
Cysteine as Cystine*	ND	ND	ND	134.8	131.4	168.3	131.7	ND	135.2	148.1	ND	0.4488
Tryptophan	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	
Phenylalanine	<25	<25	32.46	<25	<25	<25	<25	ND	<25	<25	28.5	0.1101
Isoleucine	<25	<25	<25	<25	<25	<25	<25	ND	<25	<25	<25	
Leucine	<25	47.03 de	43.01 cd	53.44 e	46.79 de	40.32 bcd	52.02 e	ND	34.37 b	37.08 bc	21.79 a	<0.001
Lysine	<25	46.13 bc	44.36 ab	50.26 d	50.08 d	47.97 cd	49.86 d	ND	49.04 cd	47.31 bcd	42.95 a	<0.001
Proline	2033 abc	1967 ab	2063 bc	1942 ab	3119 f	1968 ab	2128 c	1911 a	1934 ab	2290 d	2431 e	<0.001

Table 21 Amino Acids profile Gifford's Creek expressed in µmol/L - Wine

GC Vineyard Wine/ µmol/L	CTR	Asp	Ser	Gln	Arg	Ala	Glu	GSH	DAP	BLEND1	BLEND2	P*
Aspartic Acid	<25	**<25**	32.03 a	<25	<25	<25	<25	<25	73.83 b	<25	<25	0.005
Glutamic Acid	152.8 a	194.1 b	279 d	219.8 c	170.4 a	230.3 c	**438.9 f**	168.3 a	404.6 e	209.6 bc	213.6 bc	<0.001
GSH	163.9 bc	160.3 abc	164.5 bc	175.9 c	168 bc	159.5 abc	153.1 ab	**576.7 e**	154.1 ab	142.1 a	218.3 d	<0.001
Asparagine	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	<0.001
Serine	31.46 a	35.55 bc	**119.4 f**	37.17 bcd	37.57 bcde	37.33 bcde	39.39 de	34.72 ab	183.8 g	41.27 e	39.28 cde	<0.001
Glutamine	283.6 a	360.7 c	579.4 f	**516.2 e**	307.5 ab	424.7 d	425.7 d	276.7 a	1198 g	342.1 bc	350.1 c	<0.001
Histadine	65.36 a	72.12 ab	72.35 ab	76.53 b	76.9 b	74.23 b	73.82 b	193.2 d	99.23 c	78.55 b	105.3 c	<0.001
Glycine	106.7 ab	122.9 c	132.3 d	121.8 c	106.5 ab	124.5 cd	123.2 c	102.8 a	190.8 e	110.6 ab	113.1 b	<0.001
Threonine	<25	<25	29.8	<25	<25	<25	<25	<25	97.43	<25	<25	0.01267
Arginine	1688 a	2064 bc	1843 ab	2096 bc	**2325 c**	2063 bc	1886 ab	1662 a	3245 d	1989 b	2008 b	<0.001
Alanine	346.2 a	503.5 bc	943 e	584.2 c	**395.2 ab**	1063 e	760.1 d	373.5 ab	1855 f	535.2 c	538.8 c	<0.001
GABA	<25	<25	<25	<25	<25	<25	<25	<25	<25	<25	<25	
Tyrosine	<25	<25	27.73	25.84	<25	25.43	26.9	<25	35.98	<25	28.61	0.4069
Valine	<25	<25	35.7 a	27 a	<25	28.69 a	<25	<25	91.28 b	<25	36.43 a	0.0024
Methionine	<25	26.51	<25	<25	<25	<25	<25	<25	<25	<25	<25	0.55216
Cystine	ND	ND	ND	<25	<25	<25	<25	<25	<25	<25	<25	
Cysteine as Cystine*	ND	ND	176 a	259.8 b	144.1 ab	158.2 ab	136 ab	158.4 ab	449.7 c	176.9 ab	183 ab	0.02213
Tryptophan	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	
Phenylalanine	<25	<25	<25	<25	<25	<25	<25	<25	27.27	<25	<25	
Isoleucine	<25	<25	<25	<25	<25	<25	<25	<25	<25	<25	<25	
Leucine	131.7 b	125.9 b	<25	<25	<25	<25	<25	<25	<25	<25	30.76 a	<0.001
Lysine	43.07 bc	43.2 bc	43.79 bc	43.63 bc	42.36 abc	44.16 c	42.85 bc	39.91 a	52.39 d	41.39 ab	44.58 c	<0.001
Proline	2669 bc	2651 bc	2768 bc	2820 bcd	3001 de	3103 e	3026 de	2799 bcd	2217 a	2594 b	2824 cd	<0.001

7.0 Conclusion

In this study, with the addition of 7 different amino acids 2 blend of amino acids and a usual DAP winery product, it was found that the aromatic compounds change in the wine, this result also depends on the initial YAN of the must of Sauvignon Blanc.

The addition of amino acids produced differences in fermentation kinetics. It should be noted that in the OV vineyard the ferments were blocked on the 27th day by not reaching the end of the desired alcoholic fermentation.

Alanine has the lowest reducing sugar (8 g / L) related to a higher alcohol content. CTR and GSH treatments have higher reducing sugars corresponding to lower concentrations of ethanol.

For the GC vineyard there are no significant differences in ethanol concentrations, all the ferments reach total sugar consumption in 14 days with the exception of DAP which achieves this result in 11 days.

In the two different vineyards it turns out that in Omaka Vineyard with Low YAN a high concentration of single amino acids was added in the juice instead in Gifford's Creek Vineyard with High YAN a low concentration of single amino acids was added in the must.

As a consequence, all treatments in Omaka Vineyard gave significant differences in the aromatic compounds, Serina developed the highest concentration of Alcohols, Alanine produced a high concentration of Esters comparing with the other treatments, for the Tioli did not significant differences are developed.

In Gifford's Creek there were no major significant differences, BLEND1 developed a higher concentration of esters.

For the DAP treatment, a greater concentration was added, erroneously, instead making a lower concentration of aromatic compounds.

For the sensory analysis of the wine carried out in an unofficial manner, 66 samples were taken, 33 samples for Omaka Vineyard and 33 samples for Gifford's Creek Vineyard, the result of this research is not statistically analysable, there was a preference to treatment with Alanina and Serina for Omaka Vineyard, instead for Gifford's Creek Vineyard there was not a majority in the choice because the wines were similar in the sense of smell but with a preference for BLEND1.

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Attachments

Influences of the amino acids during alcoholic fermentation and development of aroma compounds in Sauvignon Blanc

Background:

The development of robust small-scale winemaking protocols is essential for reliable, repeatable wine research. The composition and concentration of nitrogenous compounds in juice affects yeast metabolism and can influence the rate of fermentation and the production of flavour and aroma compounds (particularly varietal thiols) in Sauvignon blanc wine. Yeast (*Saccharomyces cerevisiae*) preferentially use nitrogen sources such as ammonium ions and free alpha amino nitrogen compounds, present in the form of primary amino acids. Together ammonium and amino acid concentration in the must (referred to as yeast assimilable nitrogen (YAN)) can largely determine yeast cell population or biomass yield, fermentation rate and duration. There are seven amino acids identified as being yeast preferred. These amino acids along with ammonium are the preferred source of nitrogen for yeast during fermentation.

To achieve consistent fermentation rates and reduce variation between replicate research scale ferments it is important to understand the impact of juice nitrogen/nutritional status on fermentation rate and wine composition. This project aims to build on study a carried out by Lukas Hermann in the 2016 vintage which explored the influence of juice inoculation rate and yeast nutrition on fermentation rates, thiol production and composition of Sauvignon blanc wine. In early 2017 Alessandro Serughetti, will undertake a study to determine the influence of juice nitrogen/nutritional status by way of amino acid additions in addition to, or in place of, the standard diammonium phosphate (DAP) additions on fermentation rates, thiol production and Sauvignon blanc wine composition.

Objectives:

- To investigate the effect of differing juice nitrogen/nutritional status on fermentation rates and the impact on flavour and aroma profiles in the wine on a range of Sauvignon blanc juices.
- Determine the extent to which types of juice nitrogen supplements (concentration and composition) wine composition at microvin fermentation scale (700ml volume) using adapted Plant & Food Research (PFR) micro-vinification Sauvignon blanc winemaking protocols.
- To determine suitability and develop specifications for the optimum use of amino acid additions to manipulate juice nitrogen/yeast nutrition levels for use in

research scale winemaking protocols for the New Zealand Grape and Wine Research Programme.

Material and Methods

Sauvignon blanc juice samples obtained from existing project work will be fermented to established protocols with differing addition rates of yeast preferred amino acids in a factorial experimental design. Fermentations will be carried out at 700 mL scale and replicated three times for each treatment. Rate calculations and more detailed experimental design to be undertaken by the student.

Juices will undergo detailed chemical analyses including:

Total Soluble Solids, Titratable Acidity, pH, Free and Total SO2, Primary Amino Acids (nitrogen by o-phthaldialdehyde assay - NOPA), Ammonium and Yeast Available Nitrogen, Optical densities @ 280, 320 and 420nm, Glucose, Fructose and Sucrose, Major Organic Acids (by High Performance Liquid Chromatography), Major Cations (by Capillary Electrophoresis) and Primary Amino Acids (by High Performance Liquid Chromatography) and if available Green Leaf Volatile compounds (Gas Chromatography with Mass Spectrophotometry)

Post fermentation analyses will include:

% alcohol, Titratable Acidity, pH and reducing sugars, Free and Total SO2, Optical densities @ 280, 320 and 420nm, Primary Amino Acids (by High Performance Liquid Chromatography), Foss FT2 Winescan spectral profile and Volatile Thiols.

Plant & Food Research 20L

Sauvignon Blanc Winemaking Protocol 2017

1. Hand harvest 40kg fruit for each treatment from replicated vineyard plots. No field additions of sulphur.
2. Transfer to winery, crush and destem. Add PMS at 80g/T (40ppm SO_2).
3. Add enzyme (50ml/tonne Rapidase Clear (liquid)) to crushed/destemmed grapes.
4. Transfer to 40L containers under CO_2 cover. Give 1 hour skin contact time at 6˚C under CO_2 cover.
5. Transfer to 20L hydro press. Collect free run under CO_2 cover. Press off under CO_2 cover. Pressing regime:
    i. Press at 2.0 bar 3 min.
    ii. Press at 4.0 bar for 14 min.
6. Add 0.5g/L of bentonite Seporit PORE-TEC (ERBSLÒH) to pressed juice (addition rate as per manufacturers recommendations is 50-150 g/100L. Mix in 5 fold the quantity of water and leave to swell for at least 4 hours or overnight).
7. Cold settle juice for 24 hours at 6°C.
8. Rack off solids under CO_2 cover and rack a proportion of fluffy lees to add back to juice for turbidity adjustment. Adjust juice turbidity to between 120 - 150NTU with fluffy lees checking after each addition with the turbidity meter. Transfer juice to 700ml fermentation vessels.
9. Take 2 x microtubes and 2 x 50ml juice samples and freeze (1 sample in -50 & one in -20˚C). 1 x 50ml falcon tube for juice analysis (Brix, pH, TA, winescan, OD280, OD320, OD420, free & total SO_2, reducing sugars, Total PAA, ammonium, amino acids, organic acids, cations, on fresh juice.
10. Warm juice to 15˚C and inoculate with X5 yeast (250mg/L), rehydrate yeast using standard procedure.

Standard Yeast hydration procedure:

- Heat water bath to 35-42˚C

- Put required amount of distilled water in beaker and heat in water bath.
- Check water temp with thermometer and when 32- 35˚C add yeast. Don't stir.
- After 20 mins in water bath stir, remove and sit on bench for 15 mins. Put in bath of water in chiller to acclimatise to chiller temp.
- Inoculate ferments

11. DAP and Superfood® additions are made when YAN concentrations are below 250ppm to make YAN's up to a standard level of 250ppm. 50% of the YAN requirement is made using DAP and 50% using Superfood®. DAP and superfood additions made 1 day after inoculation.
12. Ferment at 15°C and aim for ferment temperatures of 14.5-15°C.
13. Ferment to dryness. Residual sugar ≤ 2g/L
14. Stop fermentation with 50ppm sulphur addition seal and settle.
15. Leave to settle for 24 hours.
16. Fill 50ml falcon tubes and centrifuge. (4600rpm for 5 mins).
17. Fill 2 x 35ml and microtubes and freeze at -50°C. Take 2 x 50ml falcon tubes for primary wine analysis.